Customized PTO/SB/05

UTILITY PATENT APPLICATION TRANSMITTAL *(under 37 CFR 1.53(b))*	Docket No.	P06282US02/BAS
	1st Inventor	FOSTER et al.
	Title	S. AUREUS FIBRINOGEN BINDING PROTEIN

TO: ASSISTANT COMMISSIONER FOR PATENTS
BOX PATENT APPLICATION
Washington, D.C. 20231

APPLICATION ELEMENTS

- X Fee Transmittal (see FEE CALCULATION below)
- X Specification [total pages = 27]
- X Drawings:
  - X total sheets = 13
  - ____ there are no drawings
- ____ Oath or Declaration: [total pages = 1]
  - ____ Newly executed (original or copy)
  - X Copy from a prior application. *(for cont./div. appln.)*

ACCOMPANYING APPLICATION PARTS

- ____ Assignment Papers (cover sheet and document(s))
- ____ Information Disclosure Statement (IDS) incl. PTO-1449
- X Preliminary Amendment
- X Return Receipt Postcard
- ____ Certified Copy of Priority Document
- X Other: **Letter Requesting Transfer of Prior Sequence Information**
- ____ Sequence Listing

X **CONTINUING APPLICATION**

This is a ____ Continuation X Divisional ____ Continuation-in-Part of: Prior Application No.: 09/421,868 Examiner: Graser Group/Art Unit: 1641

FOR CONTINUATION or DIVISIONAL APPLICATIONS ONLY: The entire disclosure of the prior application, from which an oath or declaration is supplied above, is considered a part of the disclosure of the accompanying continuation or divisional application and is hereby incorporated by reference. The incorporation can only be relied upon when a portion has been inadvertently omitted for the submitted application parts.

FEE CALCULATION and notations

	NOW	*Basic Number*	*Present Extra*	*Rate*	$
____ TOTAL CLAIMS	7	- 20	0	X $ 18 =	
____ INDEP. CLAIMS	5	- 3	2	X $ 80 =	160
____ MULTIPLE DEPENDENT CLAIM(S)				+ $ 270 =	
X			BASIC FEE	$ 710 =	710
			TOTAL OF ABOVE CALCULATIONS =		870
____ Reduction by ½ for **small entity status** of applicant				–	
			SUBTOTAL =		870
____ Fee for recording of assignment				+ $ 40 =	
			TOTAL OF ALL FEES =		**870.00**

____ No check is enclosed, and no charge should be made to our account.

X A check in the amount of $870.00 is enclosed. If no check or an insufficient check is enclosed and a fee is due in connection herewith, the Commissioner is authorized to charge any fee or additional fee due in connection herewith to Deposit Account No. 12-0555. A duplicate of this sheet is enclosed.

CORRESPONDENCE ADDRESS

X Customer Number: **00881**

Responsible Attorney: **B. Aaron Schulman**
Registration No.: 31,877

Signature: B. Aaron Schulman

Date: 5 October 2000

Filing Attorney: B. Aaron Schulman
Telephone: 703-739-4900

Registration No.: 31,877
Facsimile No.: 703-739-9577

LARSON & TAYLOR, PLC • 1199 North Fairfax St. • Suite 900 • Alexandria, VA 22314

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Patent

In re patent application of: FOSTER et al.

Serial No.: New U.S. Application

(Divisional of USSN 09/421,868)

Filed: Herewith

For: S. AUREUS FIBRINOGEN BINDING PROTEIN

Examiner: Graser

Art Unit: 1641

Docket No.: P06282US02/BAS

PRELIMINARY AMENDMENT

Honorable Assistant Commissioner of Patents
Washington, D.C.

S I R:

Prior to examination, please amend the above-identified application as follows.

IN THE SPECIFICATION:

Page 1, prior to the first line, please insert:

--This is a Divisional of Application No. 09/421,868, filed October 19, 1999, which was a divisional application of Application No. 08/293,728, filed August 22, 1994, now U.S. Patent No. 6,008,341.--

Page 4, line 5, after "in Figure 2" insert --and Sequence ID No. 1--;

line 20, after "Figure 2" insert --and Sequence ID No. 1--.

Page 5, lines 27-33, please delete entirely;

line 35, delete "Figure 5" and insert --Figure 4--.

Page 6, line 4, delete "Figure 6" and insert --Figure 5--;

line 19, delete "Figure 7" and insert --Figure 6--;

lines 33-39, please delete entirely.

Page 7, lines 1-3, please delete entirely;

line 5, delete "Figure 10" and insert --Figure 7--;

line 13, delete "Figure 11" and insert --Figures 8A-B--;

line 21, delete "Figure 12" and insert --Figures 9A-B--.

Page 8, line 32, after "at the", please insert --NCIMB, Aberdeen, Scotland--.

line 33, after "on", please insert --July 2, 1998--.

line 33, after "Accession No.", please insert --NCIMB40959--.

Page 9, line 22, after "KpnI fragment", please insert --which is contained in plasmid pCF10 which was deposited at the National Collections of Industrial and Marine Bacteria, Ltd., Aberdeen, Scotland, in September, 1994, and which was accorded Accession No. 40674--.

line 26, after "Figure 2A" insert --and Sequence ID No. 1--;

line 28, after "97,058 Da" insert --, see Sequence ID Nos. 1 and 2--;

line 30, after "2B" insert --and Sequence ID No. 2--.

Page 10, line 21, delete "(Figure 4, lane 1)";

lines 28-29, delete "(Figure 4, lanes 2 and 3)".

Page 11, line 19, delete "(Figure 5)" and insert --(Figure 4)--;

line 21, delete "(Figure 5)" and insert --(Figure 4)--;

line 23, delete "(Figure 5)" and insert --(Figure 4)--;

line 31, delete "Figure 6)" and insert --Figure 5)--;

line 35, delete "(Figure 6)" and insert --(Figure 5)--;

line 37, delete "Figure 6") and insert --Figure 6)--;

line 39, delete "(Figure 7A)" and insert --(Figure 6)--.

Page 12, line 2, delete "(Figure 6)" and insert --(Figure 5)--;

line 5, delete "(Figure 7A)" and insert --(Figure 6)--;

line 7, delete "and Figure 7A)" and insert --and Figure 6)--;

line 9, after "(SDSDSDSDSDSDGGGC" insert --, Sequence ID No. 16)--;

line 30, delete "(Figure 6)" and insert --(Figure 5)--;

line 34, delete "(Figure 6)" and insert --(Figure 5)--;

line 38, delete "(Figure 7B)" and insert --(Figure 6)--.

Page 13, line 37, delete "(Figure 5)" and insert --(Figure 4)--.

Page 14, line 7, delete "(Figure 8, lane 2)";

line 10, delete "(Figure 8, lane 3)";

line 17, delete "(Figure 8, lane 4)".

Page 15, line 17, delete "(Figure 9)";

line 36, delete "(Figure 10)" and insert --(Figure 7)--;

line 38, delete "(Figure 10)" and insert --(Figure 7)--.

Page 16, line 10, delete "(Figure 11)" and insert --(Figures 8A-B)--;

lines 14-15, delete "(Figure 12)" and insert --(Figures 9A-B)--.

Page 19, line 8, after "VGTLIGFGLL" insert --, Sequence ID No. 17--;

line 9, after "GKIIGID" insert --, Sequence ID No. 18--

line 10, after "MNQTSNETTFNDTNTV" insert --, Sequence ID No. 19--;

line 11, after "AVAADAPAAGTDITNQLT" insert --, Sequence ID No. 20--.

After page 26, please insert the attached sequence listing originally filed August 24, 1998 in Application No. 08/293,728, the grandparent to the present application.

REMARKS

The above changes of the type that were previously made in the parent cases to the present application in order to overcome various objections.

In addition, pursuant to MPEP 2422.05, Applicants are filing herewith a letter requesting transfer of the previously filed sequence information, and are filing the present amendment to insert that sequence listing in the present application. As indicated in the attached letter, the paper copy of the sequence listing attached hereto is identical to the CRF of the grandparent case.

Favorable consideration of the amended application is respectfully requested.

Respectfully submitted,

Date: 10/5/00

By: _____
B. Aaron Schulman
Registration No. 31,877

LARSON & TAYLOR, PLC
Transpotomac Plaza
1199 North Fairfax Street, Suite 900
Alexandria, Virginia 22314
(703) 739-4900

THE S. AUREUS FIBRINOGEN BINDING PROTEIN GENE

Field of the Invention

The invention relates to the isolation of the fibrinogen binding protein gene from Staphylococcus aureus and to the use of the fibrinogen binding protein and antibodies generated against it for wound healing, blocking adherence to indwelling medical devices, immunisation or diagnosis of infection.

Background of the Invention

In hospitalised patents Staphylococcus aureus is an important cause of infections associated with indwelling medical devices such as catheters and prostheses (Maki, 1982; Kristinsson, 1989) and non-device related infections of surgical wounds. A recent significant increase in isolates from European and US hospitals which are resistant to several antibiotics and the potential threat of emergence of vancomycin resistance in S. aureus has reinforced the importance of developing alternative prophylactic or vaccine strategies to decrease the risk of nosocomial infections due to S. aureus.

Initial localised infections can lead to more serious invasive infections such as septicaemia and endocarditis. In infections

associated with medical devices, plastic and metal surfaces become coated with host plasma and matrix proteins such as fibrinogen and fibronectin shortly after implantation (Baier, 1977; Kochwa *et al*, 1977; Cottonaro *et al*, 1981). The ability of *S. aureus* to adhere to these proteins is believed to be a crucial determinant for initiating infection (Vaudaux *et al*, 1989, 1993). Vascular grafts, intravenous catheters, artificial heart valves and cardiac assist devices are thrombogenic and are prone to bacterial colonization. *S. aureus* is the most damaging pathogen of such infections.

Fibrin is the major component of blood clots and fibrinogen/fibrin is one of the major plasma proteins deposited on implanted biomaterial. There is considerable evidence that bacterial adherence to fibrinogen/fibrin is of importance in initiation of device related infection. (i) *S. aureus* adheres to plastic coverslips coated in vitro with fibrinogen in a dose-dependent manner (Vaudaux *et al*, 1989) and to catheters coated *in vitro* with fibrinogen (Cheung and Fischetti, 1990). (ii) The organism binds avidly via a fibrinogen bridge to platelets adhering to surfaces in a model that mimics a blood clot or damage to a heart valve (Herrmann *et al*., 1993). (iii) *S. aureus* can adhere to cultured endothelial cells via fibrinogen deposited from plasma acting as a bridge (Cheung *et al*., 1991). This suggests that fibrinogen could have a direct role in promoting invasive endocarditis. (iv) Mutants defective in a global regulatory gene *sar* have reduced adherence to fibrinogen and have reduced infectivity in a rat endocarditis infection model (Cheung *et al*., 1994). While this is indicative of a role for adherence to fibrinogen in initiating endocarditis it is by no means conclusive because *sar* mutants are pleiotropic and could also lack other relevant factors.

A receptor for fibrinogen often called the "clumping factor" is located on the surface of *S. aureus* cells (Hawiger *et al*., 1978, 1982). The interaction between bacteria and fibrinogen in solution results in instantaneous clumping of bacterial cells. The binding site for clumping factor of fibrinogen is located in the C-terminus of the gamma chain of the dimeric glycoprotein. The affinity for the fibrinogen receptor is very high (Kd 9.6×10^{-9} M) and clumping occurs in low concentrations of fibrinogen. It is assumed that clumping factor also

promotes bacterial adhesion to solid-phase fibrinogen and to fibrin.

Clumping factor has eluded previous attempts at molecular characterisation. Reports of attempts to purify clumping factor described molecules with molecular masses ranging from 14.3kDa to 420kDa (Duthie, 1954; Switalski, 1976; Davison and Sanford, 1982; Espersen et al., 1985; Usui, 1986; Chhatwal et al., 1987; Lantz et al., 1990) but none were followed up with more detailed analysis. Fibrinogen is often heavily contaminated with IgG and fibronectin and unless specific steps were taken to eliminate them these studies must be suspect.

More recently it has been shown that S. aureus releases several proteins that can bind to fibrinogen (Boden and Flock, 1989, 1992, 1994; Homonylo McGavin et al., 1993). One of these is probably the same as the broad spectrum ligand binding protein identified by Homonylo McGavin et al., (1993). Another is coagulase (Boden and Flock, 1989), a predominately extracellular protein that activates the plasma clotting activity of prothrombin. Coagulase binds prothrombin at its N-terminus and also interacts with fibrinogen at its C-terminus (McDevitt et al., 1992). However, a hypothesis that the cell-bound form of coagulase is the clumping factor was disproved when coagulase-defective mutants were shown to retain clumping factor activity (McDevitt et al., 1992). There is no evidence that the fibrinogen binding region of any of these proteins is exposed on the bacterial cell surface and consequently there is no evidence that any is clumping factor.

Object of the Invention

An object of the present invention is to obtain a minimal fibrinogen binding protein. A further objective is to obtain said protein by means of a genetic engineering technique by using e.g. a plasmid comprising a nucleotide sequence coding for said protein. A further objective is to obtain said protein by chemical synthesis. An additional objective is to generate antisera against said protein.

Summary of the Invention

The present invention relates to an isolated fibrinogen binding protein gene from S. aureus, particularly the DNA molecule having the sequence shown in Figure 2, or a substantially similar sequence also encoding S. aureus fibrinogen binding protein.

The invention also relates to hybrid DNA molecules, e.g. plasmids comprising a nucleotide sequence coding for said protein. Further the invention relates to transformed host micro-organisms comprising said molecules and their use in producing said protein. The invention also provides antisera raised against the above fibrinogen binding protein and vaccines or other pharmaceutical compositions comprising the S. aureus fibrinogen binding protein. Furthermore the invention provides diagnostic kits comprising a DNA molecule as defined above, the S. aureus fibrinogen binding protein and antisera raised against it.

By "substantially similar" is meant a DNA sequence which by virtue of the degeneracy of the genetic code is not identical with that shown in Figure 2 but still encodes the same amino-acid sequence; or a DNA sequence which encodes a different amino-acid sequence which retains fibrinogen binding protein activity either because one amino-acid is replaced with another similar amino-acid or because the change (whether it be substitution, deletion or insertion) does not affect the active site of the protein.

Drawings

The invention will be described further with reference to the drawings in which there is shown:

Figure 1. Adherence of S. aureus Newman strains to fibrinogen-coated PMMA coverslips. The number of adherent bacteria is shown as a function of fibrinogen adsorbed on the coverslip. The symbol for Newman wild type is IIIIXIII. Symbols for Newman mutant strains are as follows: mutant 1,-□-; mutant 2,-△-; mutant 3,-◇-; mutant 4,-▽-. Symbols for Newman mutants carrying pCF16 are as follows: mutant 1,-■-; mutant 2,-▲-; mutant 3,-◆-; mutant 4,-▼-.: The number of bacterial cells bound is shown as CFU (mean +/- range, n=2). In points where

range bars are not visible, the bars are smaller than the symbols.

Figure 2. (A) Nucleotide and deduced amino acid sequence of the clfA gene of Staphylococcus aureus strain Newman. The sequence has been lodged in the EMBL Data Library under the accession number Z18852 SAUCF. Putative -35, -10, ribosome binding site (RBS) and transcriptional stop regions are indicated on the nucleotide sequence. For the ClfA protein, the start of the signal peptide (S), non repeat region (A), repeat region (R), wall-spanning region (W) and membrane spanning region (M) are indicated by horizontal arrows. The LPXTG motif is underlined.
(B) Schematic diagram showing the domain organization of the ClfA protein. S, signal peptide; A, non-repeat region; R, repeat region; W, wall region;, M, membrane spanning region and +, positively charged residues. The position of the LPXTG motif is indicated.

Figure 3. Proteins purified from E. coli TB1 expressing pCF17. A DNA fragment corresponding to the N-terminal half of ClfA (residues 23-550; Region A) was generated by PCR and cloned in-frame into the expression vector pKK233-2 to generate pCF17. The N-terminal sequence was deduced for the three fibrinogen binding proteins (105kDa, 55 kDa and 42kDa) purified from an induced culture of E. coli carrying pCF17 (Table 1) and the location of each with respect to the A domain and amino acids represented are indicated. Recombinant proteins which possess fibrinogen binding activity are denoted by ++.

Figure 4. Western affinity blotting analysis of fibrinogen binding proteins from E. coli TB1 expressing pCF17. Samples were fractionated by SDS-PAGE, transferred to nitrocellulose membranes and probed with fibrinogen labelled with horseradish peroxidase (HRP). 100 micro g of E. coli pCF17 induced lysate (Lane 1), 5 micro g of proteins purified from the fibrinogen-Sepharose column and 1 micro g of purified 42kDa protein. Sizes are in kDa.

Figure 5. Inhibition of adherence of strain Newman Δ spa to fibrinogen-coated PMMA coverslips by anti-ClfA sera and preimmune sera. The symbol for anti Region A serum N2 is -■- and the symbol for preimmune serum N2 is -□-. The symbol for anti Region RWM serum C2 is -●-. The percentage inhibition is shown as mean +/- range, n=2. In

points where range bars are not visible, the bars are smaller than the symbols.

Figure 6. Localization of the fibrinogen binding domain of ClfA. DNA fragments corresponding to the different segments of clfA were generated by PCR and cloned in-frame into the fusion protein expression vector pGEX-KG. ClfA truncates were expressed as fusion proteins with glutathione S-transferase. The location of the clfA gene fragments, the amino acids represented and the length of the protein amplified are also indicated. The properties of the recombinant proteins are indicated. Proteins were assessed for (a) ability to bind to fibrinogen in the affinity blotting assay (binds fg), (b) the ability of lysates to inhibit the clumping of bacteria in soluble fibrinogen (inhibits clumping), (c) the ability of lysates to inhibit the adherence of bacteria to solid-phase fibrinogen (inhibit adherence), and (d) the ability of lysates to block neutralising antibodies (blocks Abs). ++, positive reaction; -, negative; ND, not done.

Figure 7. (A) Inhibition of adherence of S. aureus Newman to fibrinogen-coated coverslips by lysates containing ClfA truncates. Symbols are E. coli pCF24 uninduced lysate -△-, E. coli pCF24 induced lysate -▲- E. coli pCF25 uninduced lysate -□-, E. coli pCF25 induced lysate -■-. The percentage inhibition is shown as mean +/- range, n=2. In points where range bars are not visible, the bars are smaller than the symbols.
(B) Inhibition of adherence of S. aureus Newman to fibrinogen-coated coverslips by lysates containing ClfA truncates. Symbols are E. coli pCF27 lysate -■-, E. coli pCF28 lysate-●-, E. coli pCF29 lysate -▲-, E. coli pCF30 lysate -▼- , E. coli pCF31 lysate -◆-. The percentage inhibition is shown as mean +/- range, n=2. In points where range bars are not visible, the bars are smaller than the symbols.

Figure 8. Western immunoblotting of ClfA proteins.
Proteins released from the cell wall of S. aureus strains Newman (lane 3) and Newman clfA (lane 4), and proteins expressed by E. coli TB1 pCF3 (carrying the cloned clfA gene, lane 2) and by E. coli TB1 without the plasmid (lane 1), were studied by Western immunoblotting with anti-ClfA antibodies. Approximately 100 micro g of protein was loaded for each sample. Sizes are in kDa.

Figure 9. S. aureus strains were studied by immunofluorescence with anti-ClfA N2 serum. Newman Δ spa::Tc^r cells (+) and Newman Δ spa::Tc^r clfA::Tn917 cells (-).

Figure 10. Adherence of S. aureus Newman strains to PMMA coverslips coated in vitro with fibrinogen. The number of adherent bacteria is shown as a function of fibrinogen adsorbed on the coverslip. The symbols are, Newman wild type, -O- ; Newman clfA::Tn917, -●-. The number of bacterial cells bound is shown as c.f.u. (mean +/- range, n=2). In points where range bars are not visible, the bars are smaller than the symbols.

Figure 11. Adherence of S. aureus Newman strains onto segments of ex vivo polymer tubing exposed to canine blood. Adherence was tested to both ex vivo polyvinylchloride (PVC) and to ex vivo polyethylene (PE). The symbols are, Newman wild type, -O-; Newman clfA::Tn917, -●-. The number of bacterial cells bound is shown as c.f.u. (mean +/- range, n=2). In points where range bars are not visible, the bars are smaller than the symbols.

Figure 12. Adherence of S. aureus 8325-4 strains onto segments of ex vivo polymer tubing exposed to canine blood. Adherence was tested to both ex vivo polyvinylchloride (PVC) and to ex vivo polyethylene (PE). The symbols are: 8325-4 wild type, -□-; 83254 clfA::Tn917, -■-; 8325-4 clfA::Tn917 (pCF4), -⊠-. The number of bacterial cells bound is shown as c.f.u. (mean =/- range, n=2). In points where range bars are not visible, the bars are smaller than the symbols.

Cloning and sequencing the clumping factor gene

In view of the difficulties mentioned above with (i) obtaining pure fibrinogen, (ii) the discrepancies in reported molecular weight of "clumping factor" and (iii) the diversity of different fibrinogen binding proteins, a different approach was taken to identify the clumping factor gene involving isolating insertion mutants that inactivated the clumping phenotype. This has been described in detail by McDevitt et al., (1994).

Transposon Tn917 (Tomich et al., 1980) was used to generate insertion mutants that eliminated the fibrinogen clumping phenotype of S. aureus strain Newman. The temperature sensitive plasmid pTV1ts which carries Tn917 (Youngman, 1985) was transferred into strain Newman and several transposon insertion banks were isolated by growing cultures at 43° in broth containing erythromycin (to select for Tn917 after plasmid elimination). Cultures of the banks were mixed with fibrinogen, the agglutinated cells were removed and the surviving cells in the supernatants were screened for clumping factor-deficient mutants. Four mutants were isolated from separate banks. The Tn917 elements were transduced into a wild-type Newman host with phage 85. In each case all the transductants screened had inherited the clumping factor deficiency proving that the Tn917 insertions caused the mutant phenotypes. The clumping factor mutants expressed the same level of coagulase as the wild-type strain, further supporting the conclusion that clumping factor and coagulase are distinct entities.

The mutants were analyzed by Southern hybridization using an internal fragment of Tn917 as a probe in order to identify HindIII junction fragments comprising transposon and flanking chromosomal sequences. A junction fragment from one mutant was cloned using standard techniques in plasmid vector pUC18 (Yanisch Perron et al., 1985). A fragment comprising only chromosomal DNA flanking the transposon was isolated from this plasmid and used in turn as a probe in a Southern blot of genomic DNA of Newman wild-type and each of the mutants. A HindIII fragment of 7kb that hybridized in Newman wild-type was missing in each of the mutants. Genomic DNA of Newman wild-type was cleaved with HindIII and ligated with plasmid pUC18 cut with the same enzyme and transformed into E. coli TBI (Yanisch-Perron et al., 1985). Transformants were screened by colony hybridisation using the junction fragment probe. Plasmid pCF3 (pUC18 carrying the 7kb HindIII fragment) was isolated. Plasmid pCF3 was deposited at the on under the Accession No. , such deposit complying with the terms of the Budapest Treaty.

The 7kb HindIII fragment was subcloned into pCL84, a single copy non-replicating vector which integrates into the chromosome of S. aureus (Lee et al., 1991), forming pCF16. pCF16 was transformed into S. aureus strain CYL316 (Lee et al., 1991) selecting for tetracycline

resistance. The integrated plasmid was then transduced with phage 85 into each of the Newman clf mutants. In a microtitre clumping assay the Newman mutants were completely devoid of activity even at the highest concentrations of fibrinogen, whereas the wild-type had a titre of 2048 and could interact productively with very low concentrations of fibrinogen. The integrated single copy plasmid pCF16 restored the clumping activity of each of the mutants to the same level as that of the parental strain. Thus the HindIII fragment must express a functional protein which complements the clumping deficiency of the mutants.

S. aureus Newman adhered to solid-phase fibrinogen coated onto polymethylmethacrylate (PMMA) coverslips in a concentration dependent manner (Figure 1). Each clf mutant showed drastic reduction in adherence. This was restored to the level of the parental strain by pCF16. This data shows that the ability of Newman to form clumps in soluble fibrinogen correlates with bacterial adherence to solid-phase fibrinogen.

Fragments from the 7kb HindIII fragment in pCF3 were subcloned into pGEM7 Zf(+) (Promega). The smallest fragment which still expressed the fibrinogen binding activity was a 3.5kb HindIII-KpnI fragment. The DNA sequence of this fragment was obtained using standard techniques and has been lodged in the EMBL Data Library under the accession number Z18852 SAUCF. A single open reading frame of 2799 bp was identified (Figure 2A). The orf is called clfA and the gene product the ClfA protein. The predicted protein is composed of 933 amino acids (molecular weight 97,058 Da). A putative signal sequence of 39 residues was predicted. The predicted molecular weight of the mature protein is 92kDa. Following the signal sequence is a region of 520 residues (Region A) which precedes a 308 residue region (region R) comprising 154 repeats of the dipeptide serine-aspartate (Figure 2A and 2B). The C terminus of ClfA has features present in surface proteins of other Gram positive bacteria (Schneewind et al., 1993) that are responsible for anchoring the protein to the cell wall and membrane: (i) residues at the extreme C-terminus that are predominantly positively charged, (ii) a hydrophobic region which probably spans the cytoplasmic membrane and (iii) the sequence LPDTG which is homologous to the consensus sequence LPXTG that occurs in all wall-associated

proteins of Gram positive bacteria. This strongly suggests that ClfA is a wall-associated protein and that the N terminal part is exposed on the cell surface.

It is not obvious from the primary structure of ClfA or by comparison of ClfA with other ligand binding proteins of S. aureus (fibronectin binding protein, Signas et al., 1989; collagen binding protein, Patti et al., 1992) which part of ClfA interacts with fibrinogen.

Results

(1) Purifying the N-terminal half of the fibrinogen receptor (ClfA)

A DNA fragment corresponding to the N-terminal half of ClfA (residues 23-550; Region A) was generated by polymerase chain reaction (PCR) and cloned in-frame into the expression vector pKK233-2 (Amann and Brosius, 1985) to generate pCF17 (Figure 3). Expression of recombinant Region A was induced by adding isopropyl Beta-D-thiogalactoside (IPTG) to exponential cultures. Induced cultures contained two proteins of 105kDa and 55kDa which reacted with fibrinogen in a Western ligand blotting assay (Figure 4, lane 1). A fibrinogen-Sepharose 4B column was made by the method recommended by the manufacturer (Pharmacia). A sample of an induced culture containing these fibrinogen binding proteins was passed into the fibrinogen Sepharose 4B column. Four proteins were eluted: -105kDa, 55kDa, 42kDa and 75kDa (trace amounts). In a separate purification experiment, the 42kDa protein was purified to homogeneity. Only the 105kDa, 55kDa and 42kDa proteins bound to fibrinogen in the Western ligand blotting assay (Figure 4, lanes 2 and 3). The N-terminal sequence of these proteins was determined (Table 1). The 75kDa protein was present in trace amounts (1-2pmoles) and is not related to ClfA. The three predominant proteins bound to fibrinogen in the Western blotting assay and are related to the region A (see Figure 3). The 105kDa protein represents the intact Region A while the 55kDa and 42kDa proteins are breakdown products. The apparent molecular weights of the native region A and breakdown products of region A are much higher than that predicted from the DNA sequence (Table 1).

(2) Antibodies to the Region A of the ClfA protein (residues 23-550)

A rabbit was immunised with 30 micro g of a mixture of the 105kDa, 75kDa, 55kDa and 42kDa proteins along with Freund's complete adjuvant. The immune sera was called N2. One rabbit was also immunised with 18 micro g of the purified 42kDa ClfA truncate and the immune serum for this was called N3. Bacterial interaction with fibrinogen can be measured by a quantitative clumping titration assay (Switalski, 1976). In this assay, doubling dilutions of a fibrinogen solution (1mg/ml) are mixed in a microtitre dish with a suspension of 2 X 10^7 cells for 5 min with gentle shaking. A standard clumping concentration of fibrinogen was defined as 2X the titre. To this was added varying amounts of the anti-ClfA serum to measure the minimum inhibitory concentration that stops the clumping reaction (Table 2). Both N2 and N3 sera were potent inhibitors of the clumping of bacteria. Preimmune sera did not inhibit the clumping of bacteria. N2 sera also had a potent inhibitory activity on bacterial adhesion to surface-bound fibrinogen in the coverslip assay (McDevitt et al., 1992, 1994), expressing 95% inhibition at 1 micro g protein/ml (Figure 5). Preimmune sera did not have any inhibitory activity even at a protein concentration of 100 micro g/ml (Figure 5). In addition, antisera raised against regions R, W and M (C2) (see section 4 below) failed to inhibit adherence even at 100 micro g/ml (Figure 5).

(3) Localisation of the fibrinogen binding domain of the ClfA protein

DNA fragments corresponding to the Region A of ClfA (residues 23-550) and C terminal regions R,W and M (residues 546-933) were generated by PCR (standard conditions,) and cloned in-frame into the fusion protein expression vector pGEX-KG (Guan and Dixon, 1991) to generate pCF24 and pCF25 respectively (see Figure 6). These ClfA truncates were expressed as fusion proteins with glutathione S-transferase. An induced lysate of E. coli pCF24 (residues 23-550) expressed a fusion protein that bound to fibrinogen in a Western affinity blotting assay with peroxidase labelled fibrinogen (Figure 6). In addition, this lysate inhibited the clumping of bacteria with soluble fibrinogen in the clumping assay (Table 3 and Figure 6) and also inhibited the adherence of bacteria to immobilised fibrinogen in the coverslip assay in a dose dependent fashion (Figure 7A). A lysate of E. coli pCF25 (residues

546-933) induced with IPTG expressed a fusion protein that failed to bind to fibrinogen in the Western blotting assay (Figure 6). In addition, this lysate did not inhibit the clumping of bacteria in the clumping assay (Table 3) and did not inhibit adherence to immobilized fibrinogen in the adherence assay (Figure 7A). Uninduced lysates from both pCF24 and pCF25 failed to inhibit both clumping and adherence (Table 3 and Figure 7A).

A synthetic peptide (SDSDSDSDSDSDGGGC) designed to mimic the C-terminal repeat region of ClfA failed to inhibit the clumping of bacteria in the clumping assay when up to 107 micro g/ml was tested. In addition, the synthetic peptide failed to inhibit the adherence of bacteria in the adherence assay even at a concentration of 100 micro g/ml. Taken together, this data suggests that the fibrinogen binding domain of ClfA is located in the A domain rather than in the regions R, M, and W. It confirms the data in Table 1 which dealt with purifying fibrinogen binding proteins expressed from pCF17 and also suggests that an N-terminal ClfA protein can act both as a potent inhibitor of cell clumping in fibrinogen and also as a potent inhibitor of the adherence of bacteria to fibrinogen coated surfaces.

The fibrinogen binding domain was further localised within region A. Segments of region A were amplified by PCR and cloned into the pGEX-KG vector. Lysates from IPTG-induced cultures were examined for the presence of fibrinogen binding fusion proteins, for the ability to inhibit the clumping of bacteria in the fibrinogen clumping assay and for the ability to inhibit adherence to immobilised fibrinogen in the adherence assay. The fusion protein of pCF31 (residues 221-550) was the smallest truncate that still expressed a fibrinogen binding activity (Figure 6). It is almost identical in composition to the purified 42kDa protein (residues 219-550) described above. The fusion proteins from pCF27, pCF28, pCF29 and pCF30 all failed to bind to fibrinogen in the Western affinity blotting assay, despite reacting with antibodies generated against the A domain of ClfA (Figure 6). In addition, a lysate containing the fusion protein expressed by pCF31 was the only one to inhibit the fibrinogen clumping reaction (Table 3) and to inhibit the adherence of bacteria to immobilised fibrinogen in the adherence assay (Figure 7B). These results suggest that the fibrinogen binding site is quite extensive or that its correct conformation is

determined by flanking sequences.

An antibody neutralisation assay was adopted to help localise further the active site within residues 221-550. This assay was conducted with a protein A negative deletion mutant of S. aureus strain Newman (Patel et al., 1987) to prevent non specific reaction with IgG. Polyclonal antibodies raised against the A region of ClfA (N2) inhibited the clumping of bacteria in soluble fibrinogen (see section 2 above). In the standard clumping assay with the clumping concentration at 2X the titre, the concentration of lysates that blocked the inhibitory activity of 4.68 micro g of serum (2X the inhibitory concentration, Table 2) was determined. The lysates containing ClfA fusion proteins were assayed for their ability to neutralise the inhibiting activity of the antibodies. Truncates containing the active site might be able to adsorb out antibodies generated against the active site and thus neutralise the blocking effect on cell clumping. The lysates containing proteins expressed by pCF24 and pCF31 neutralised the inhibiting activity of the antibodies while a lysate containing the fusion protein expressed by pCF25 (Region R,W and M) did not inhibit (Table 4). Lysates containing small fusion proteins expressed by pCF30 were able to neutralise the inhibiting activity of antibodies while lysates containing fusion proteins expressed by pCF27 and pCF29 had no such activity (Table 4). Taken together this suggested that the active site is located in a 218 residue region between residues 332 and 550.

(4) Antibodies to the C-terminal half of the ClfA protein (residues 546-933)

The fusion protein present in a lysate of E. coli pCF25 (residues 546-933) induced with IPTG was purified to homogeneity by using glutathione sepharose-affinity chromatography as described by Guan and Dixon, (1991). A rabbit was immunised with 20 micro g of the fusion protein along with Freund's complete adjuvant. The immune sera was called C2. This serum failed to inhibit the clumping of bacteria in the clumping assay (Table 2) and also failed to inhibit bacterial adhesion to surface bound fibrinogen in the coverslip assay even at 100 micro g/ml (Figure 5).

(5) Identification of the native fibrinogen receptor

Proteins released from the cell wall of S. aureus strains and a lysate of E. coli expressing the cloned clfA gene were studied by Western immunoblotting with anti ClfA antibodies in order to identify ClfA protein(s). A lysate of E. coli TB1 (pCF3) (carrying the cloned clfA gene) contained several immunoreactive proteins (Figure 8, lane 2). The largest of these was ca. 190kDa. The smaller proteins are probably derivatives caused by proteolysis. S. aureus strain Newman also expresses a ca. 190kDa immunoreactive protein (Figure 8, lane 3). A smaller immunoreactive protein of ca. 130kDa was also detected and is probably also caused by proteolysis. Despite the presence of protease inhibitors and studying proteins released from cells harvested at different stages in the growth cycle (from mid-exponential to late stationary), two proteins of these sizes were always present (data not shown). Both proteins were absent in extracts of the clumping factor negative transposon insertion mutant of Newman (Figure 8, lane 4) indicating that they are products of the clfA gene.

Previously we reported the size of the ClfA protein to be ca. 130kDa (McDevitt et al., 1994) in an affinity blotting assay with fibrinogen and peroxidase labelled anti-fibrinogen antibodies. Our current immunoblotting assay is much more sensitive than the affinity blotting assay. In addition, we now know that the ClfA protein is very sensitive to degradation. Indeed the predominant immunoreactive protein detected in samples from both E. coli TB1 (pCF3) and S. aureus strain Newman which have been frozen and thawed more than twice is 130kDa indicating that the ca. 190kDa protein is labile (data not shown). Thus, the ca. 130kDa protein detected in the affinity blotting assay is most probably a smaller derivative of ClfA. The apparent size of the native ClfA protein of strain Newman appears to be ca. 190kDa. This is double that predicted from the DNA sequence, but this might be due to the unusual structure and is consistent with the aberrantly high apparent molecular weight of recombinant proteins (Table 1). The recombinant N-terminal Region A protein expressed by E. coli pCF17 also had an unexpectedly high apparent molecular weight.

(6) Surface localization of the ClfA protein by immunofluorescent microscopy

Anti-ClfA region A sera (N2) was used to confirm that Region A of ClfA is exposed on the bacterial cell surface. Protein A-deficient mutants of Newman and Newman clfA::Tn917 (clumping factor transposon insertion mutant) were isolated by transducing the Δ spa::Tc^r mutation from 8325-4 Δ spa::Tc^r to strains Newman and Newman clfA::Tn917 using phage 85. Protein A-deficient mutants were used to prevent non-specific interaction with rabbit IgG. Cells from overnight cultures of strains Newman Δ spa::Tc^r and Newman Δ spa::Tc^r clfA::Tn917 were diluted to As60 = 0.6-1.0 and fixed to glass slides using gluteraldehyde. The slides were then incubated in anti-ClfA region A serum (N2, 1 in 200) followed by fluorescein conjugated swine anti-rabbit serum (Dakopatts, 1 in 40). The cells were studied for fluorescence by microscopy (Nowicki et al., 1984). Newman Δ spa::Tc^r cells fluoresced while Newman Δ spa::Tc^r clfA::Tn917 cells did not (Figure 9). This confirmed that region A of ClfA is exposed on the cell surface of wild-type Newman and that this ClfA protein is absent in the clumping factor deficient mutant.

(7) Role of the fibrinogen receptor in adherence to in vitro- and ex vivo-coated polymeric biomaterials

A mutant of strain Newman defective in the clumping factor (clfA1::Tn917) and a complemented mutant bearing pCF16 were studied for adherence properties to biomaterials coated in vitro with fibrinogen and to ex vivo biomaterial. A canine arteriovenous shunt has been developed as a model to study plasma protein adsorption onto intravenous catheters from short-term blood-biomaterial exposures and to identify host proteins promoting adhesion of Staphylococcus aureus (Vaudaux et al., 1991).

S. aureus strain Newman adheres strongly (in a concentration dependent fashion) to polymethylmethacrylate (PMMA) coverslips coated in vitro with canine fibrinogen (Figure 10). In contrast, the fibrinogen receptor mutant was significantly defective (>95%) in its ability to adhere to the canine fibrinogen coated coverslips (Figure 10). In the

ex vivo model, either polyethylene or polyvinyl chloride tubing was exposed to canine blood for 5, 15 or 60 min at a flow rate of 300 ml/min, then flushed in phosphate buffered saline (PBS), cut into 1.5 cm segments and preincubated in 0.5% albumin in PBS to prevent non-specific staphylococcal attachment. Then, each segment was incubated with 4 x 10^6 CFU/ml of [3H]thymidine-labelled S. aureus for 60 min at 37°C in an in vitro adherence assay. When compared with the wild-type strain Newman, the fibrinogen receptor mutant strain showed a strong decrease (>80%) in attachment to ex vivo polyvinyl chloride and polyethylene tubings (Figure 11). In addition, strain 8325-4 (which binds poorly to fibrinogen-coated coverslips in vitro and to the ex vivo polymer tubings) showed a significant increase in its ability to adhere to the two different ex vivo polymer tubings when complemented with a plasmid (pCF4) expressing the fibrinogen receptor gene (Figure 12).

The data shows that fibrinogen is the major plasma protein in a short-term blood material interaction to promote staphylococcal adherence and the possession of the fibrinogen receptor is a major determinant in the ability of S. aureus to adhere to ex vivo biomaterials.

(8) Role of the fibrinogen receptor in the pathogenesis of experimental endocarditis

S. aureus strain Newman, the fibrinogen receptor mutant strain of Newman (clfA::Tn917) and a fibrinogen receptor mutant complemented with the clfA+ integrating plasmid pCF16 were compared in a previously described model of experimental endocarditis (Garrison and Freedman, 1970). This rat model investigates the early events in experimental endocarditis with catheter-induced aortic vegetations (Veg). Groups of >/-8 rats were challenged with an inoculum that resulted in 90% of vegetations being colonised by the wild-type organism (ID90). Animals were injected intravenously with the same inocula of Newman clfA and Newman clfA (pCF16). Animals were killed 12 hours after inoculation and quantitative cultures of the blood, spleen and Veg were performed. Table 5 shows the percentage of rats infected.

The data show that a mutant lacking the fibrinogen receptor was significantly less able to infect the catheter-induced aortic vegetations (decrease of 49%) when compared with the wild type strain Newman. In addition, the complemented strain had restored infectivity. The fact that all three strains infected the spleens with similar numbers suggests that the presence or absence of the fibrinogen receptor interfered specifically with bacterial colonisation of the catheter-induced aortic vegetation.

This model strongly implicates the fibrinogen receptor as an important adhesin in the pathogenesis of S. aureus endocarditis and other cardiovascular infections associated with intravenous catheters, artificial heart valves and intravenous shunts.

Uses of the invention

1. The fibrinogen binding protein or fragment containing the fibrinogen binding region can be used as a vaccine to protect against human and animal infections caused by S. aureus. For example, the fibrinogen binding protein or fragment containing the fibrinogen binding region can be used as a vaccine to protect ruminants against mastitis caused by S. aureus infections.

2. Polyclonal and monoclonal antibodies raised against the fibrinogen binding protein or a fragment containing the fibrinogen binding domain can be used to immunise passively by intravenous injection against infections caused by S. aureus.

3. The fibrinogen binding protein or an active fragment can be administered locally to block S. aureus from colonising and infecting a wound.

4. The antibody against the fibrinogen binding protein can be administered locally to prevent infection of a wound.

5. The fibrinogen binding protein or an active fragment or antibodies against the fibrinogen binding protein can be used to block adherence of S. aureus to indwelling medical devices such as catheters,

replacement heart valves and cardiac assist devices.

6. The fibrinogen binding protein or an active fragment or antibodies against the fibrinogen binding protein can be used in combination with other blocking agents to protect against human and animal infections caused by S. aureus.

7. The fibrinogen binding protein can be used to diagnose bacterial infections caused by S. aureus strains. The fibrinogen binding protein can be immobilised to latex or Sepharose (Trade Mark), and sera containing antibodies are allowed to react; agglutination is then measured.

8. The fibrinogen binding protein can be used in an ELISA test.

9. DNA gene probe for the fibrinogen binding protein for ELISA tests.

10. Antibodies to the fibrinogen binding protein can be used to diagnose bacterial infections caused by S. aureus strains.

Table 1. ClfA proteins.

Protein mol. wt. apparent*	predicted@	N-terminal sequence	ClfA residues
105kDa	57kDa	VGTLIGFGLL	23-32
75kDa	ND	GKIIGID	not related
55kDa	44kDa	MNQTSNETTFNDTNTV	143-157
42kDa	36kDa	AVAADAPAAGTDITNQLT	220-237
Native ClfA			
190kDa	92kDa		

* determined from migration on SDS-PAGE and Western blotting.
@ predicted from the amino acid sequence
ND not determined.

Table 2. Inhibition of clumping with anti-ClfA sera.

Sera	Inhibiting concentration* (micro g)
N2	2.34
N3	2.34
Preimmune N2	>300.00
Preimmune N3	>300.00
C2	>300.00

* Average of 3 experiments.

Table 3. Inhibition of clumping with lysates containing truncated ClfA proteins.

Lysate	Inhibiting concentration* (micro g)
pCF24	9.37
pCF25	>300.00
pCF24 Uninduced	>300.00
pCF25 Uninduced	>300.00
pCF27	>300.00
pCF28	>300.00
pCF29	>300.00
pCF30	>300.00
pCF31	9.37

* Average of 3 experiments.

Table 4. The ability of lysates to block the inhibiting effect of anti-ClfA N2 sera on cell clumping.

Lysate	Blocking concentration* (micro g)
pCF24	1.17
pCF25	>75.00
pCF27	>75.00
pCF28	>75.00
pCF29	>75.00
pCF30	2.34
pCF31	2.34

* Average of 3 experiments.

Table 5. Experimental endocarditis

% infected	Newman	Newman *clfA*::Tn*917*	Newman *clfA*::Tn*917* pCF16 *clfA*+
vegetation	84%	43%*	83%
blood cultures	70%	30%*	50%
spleen (x log CFU/g)	3.16	3.11	3.59

* p = 0.05 when compared to other groups

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What is Claimed is:

1. An isolated antibody raised against an *S. aureus* fibrinogen binding protein produced by a microorganism expressing a DNA molecule consisting of Sequence ID No. 1 or degenerates thereof.

2. An isolated antibody according to Claim 1 wherein said antibody is a monoclonal antibody.

3. An isolated antibody according to Claim 1 wherein said antibody is a polyclonal antibody.

4. An isolated antibody raised against an *S. aureus* fibrinogen binding protein produced by a microorganism expressing a DNA molecule encoding *S. aureus* fibrinogen binding activity as deposited in plasmid pCF3 at the NCIMB in Aberdeen, Scotland under Accession No. NCIMB40959 or degenerates thereof.

5. An isolated antibody raised against an *S. aureus* fibrinogen binding protein produced by a microorganism expressing a DNA molecule encoding *S. aureus* fibrinogen binding activity as deposited in plasmid pCF10 at the NCIMB under Accession No. 40674 or degenerates thereof.

6. An isolated antibody raised against a fibrinogen binding protein comprising an amino acid sequence selected from the group consisting of amino acids 23 to 550 of Sequence ID No. 2, 332 to 550 of Sequence ID No. 2, and 332 to 425 of Sequence ID No. 2.

7. Isolated antisera raised against an *S. aureus* fibrinogen binding protein produced by a microorganism expressing a DNA molecule consisting of Sequence ID No. 1 or degenerates thereof.

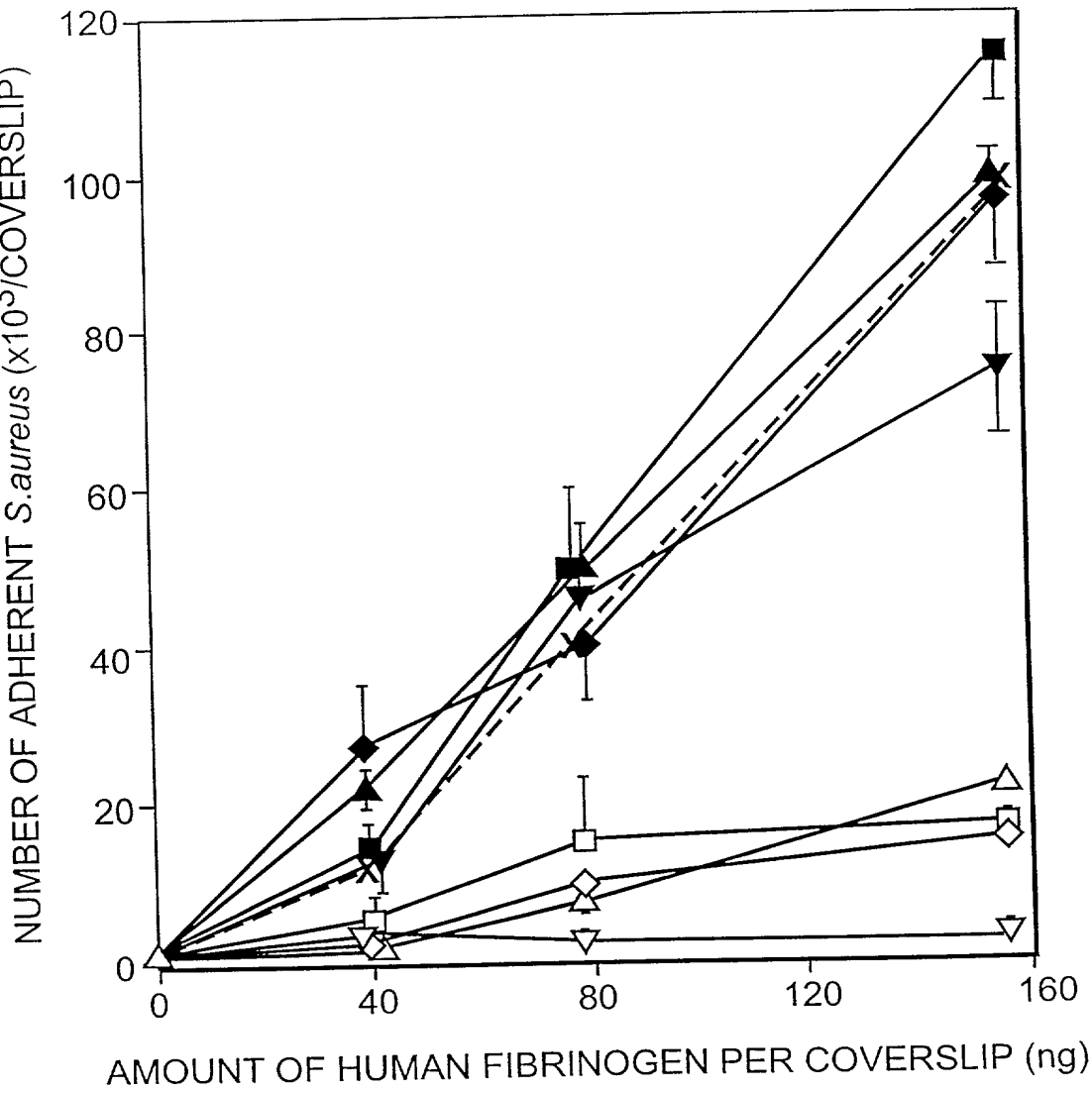
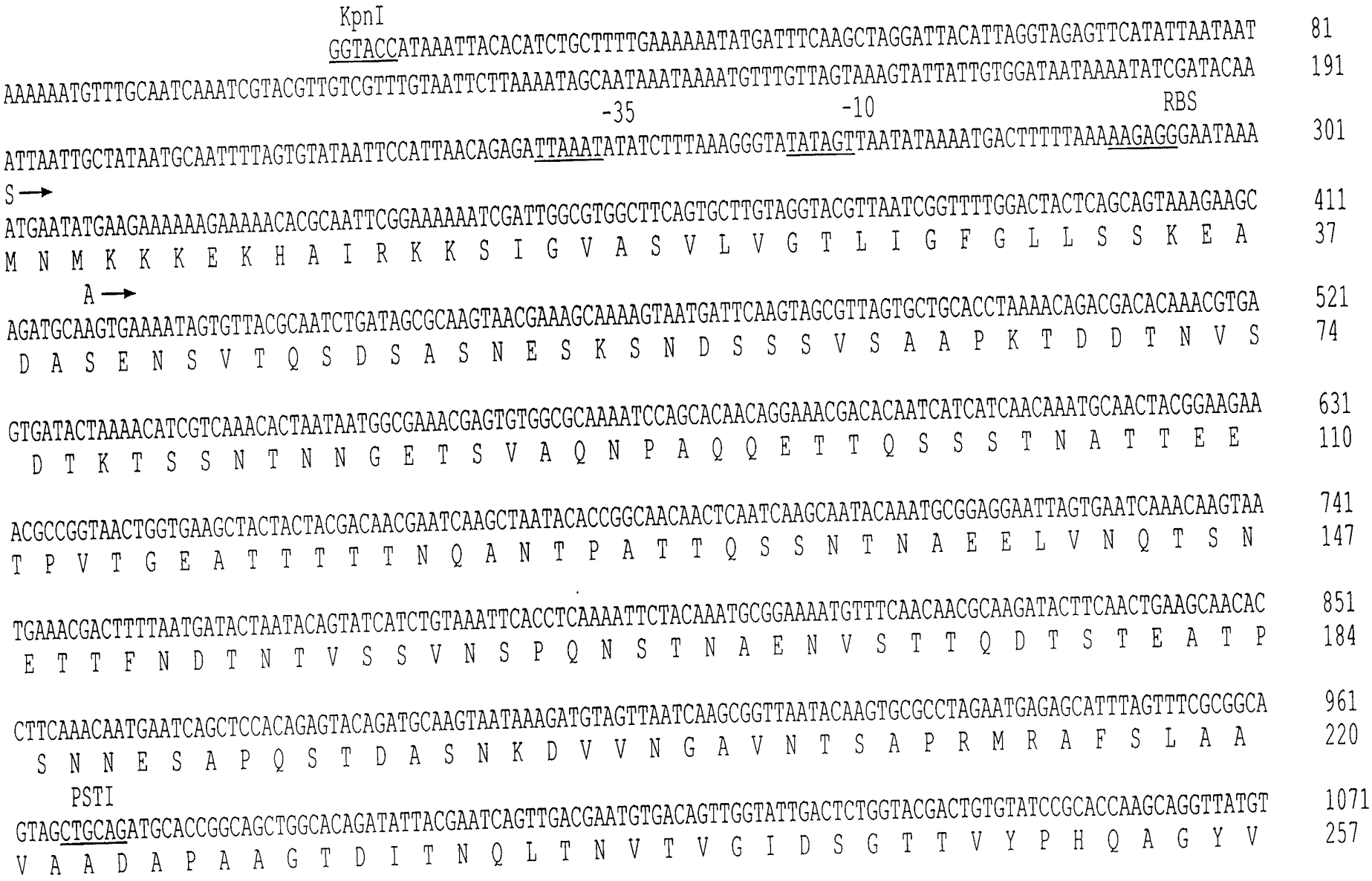

120
100
80
60
40
20
0
NUMBER OF ADHERENT S.aureus (x10^3/COVERSLIP)
0
40
80
120
160
AMOUNT OF HUMAN FIBRINOGEN PER COVERSLIP (ng)

FIG. 1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-35
-10
RBS
ATTAATTGCTATAATGCAATTTTAGTGTATAATTCCATTAACAGAGATTAAATATATCTTTAAAGGGTATATAGTTAATATAAAATGACTTTTTAAAAAGAGGGAATAAA 301
S→
ATGAATATGAAGAAAAAAGAAAAACACGCAATTCGGAAAAAATCGATTGGCGTGGCTTCAGTGCTTGTAGGTACGTTAATCGGTTTTGGACTACTCAGCAGTAAAGAAGC 411
M N M K K K E K H A I R K K S I G V A S V L V G T L I G F G L L S S K E A 37
A→
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D A S E N S V T Q S D S A S N E S K S N D S S S V S A A P K T D D T N V S 74
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D T K T S S N T N N G E T S V A Q N P A Q Q E T T Q S S S T N A T T E E 110
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E T T F N D T N T V S S V N S P Q N S T N A E N V S T T Q D T S T E A T P 184
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S N N E S A P Q S T D A S N K D V V N G A V N T S A P R M R A F S L A A 220
PSTI
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V A A D A P A A G T D I T N Q L T N V T V G I D S G T T V Y P H Q A G Y V 257

FIG. 2A-1

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 K  L  N  Y  G  F  S  V  P  N  S  A  V  K  G  D  T  F  K  I  T  V  P  K  E  L  N  L  N  G  V  T  S  T  A  K  V   294

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   P  P  I  M  A  G  D  Q  V  L  A  N  G  V  I  D  S  D  G  N  V  I  Y  T  F  T  D  Y  V  N  T  K  D  D  V  K    330

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 A  T  L  T  M  P  A  Y  I  D  P  E  N  V  K  K  T  G  N  V  T  L  A  T  G  I  G  S  T  T  A  N  K  T  V  L  V   367

AGATTATGAAAAATATGGTAAGTTTTATAACTTATCTATTAAAGGTACAATTGACCAAATCGATAAAACAAATAATACGTATCGTCAGACAATTTATGTCAATCCAAGTG   1511
 D  Y  E  K  Y  G  K  F  Y  N  L  S  I  K  G  T  I  D  Q  I  D  K  T  N  N  T  Y  R  Q  T  I  Y  V  N  P  S  G   404

GAGATAACGTTATTGCGCCGGTTTTAACAGGTAATTTAAAACCAAATACGGATAGTAATGCATTAATAGATCAGCAAAATACAAGTATTAAAGTATATAAAGTAGATAAT   1621
   D  N  V  I  A  P  V  L  T  G  N  L  K  P  N  T  D  S  N  A  L  I  D  Q  Q  N  T  S  I  K  V  Y  K  V  D  N    440

GCAGCTGATTTATCTGAAAGTTACTTTGTGAATCCAGAAAACTTTGAGGATGTCACTAATAGTGTGAATATTACATTCCCAAATCCAAATCAATATAAAGTAGAGTTTAA   1731
 A  A  D  L  S  E  S  Y  F  V  N  P  E  N  F  E  D  V  T  N  S  V  N  I  T  F  P  N  P  N  Q  Y  K  V  E  F  N   477

TACGCCTGATGATCAAATTACAACACCGTATATAGTAGTTGTTAATGGTCATATTGATCCGAATAGCAAAGGTGATTTAGCTTTACGTTCAACTTTATATGGGTATAACT   1841
   T  P  D  D  Q  I  T  T  P  Y  I  V  V  V  N  G  H  I  D  P  N  S  K  G  D  L  A  L  R  S  T  L  Y  G  Y  N  S   514

CGAATATAATTTGGCGCTCTATGTCATGGGACAACGAAGTAGCATTTAATAACGGATCAGGTTCTGGTGACGGTATCGATAAACCAGTTGTTCCTGAACAACCTGATGAG   1951
   N  I  I  W  R  S  M  S  W  D  N  E  V  A  F  N  N  G  S  G  S  G  D  G  I  D  K  P  V  V  P  E  Q  P  D  E    550
                                 R ⟶
CCTGGTGAAATTGAACCAATTCCAGAGGATTCAGATTCTGACCCAGGTTCAGATTCTGGCAGCGATTCTAATTCAGATAGCGGTTCAGATTCGGGTAGTGATTCTACATC   2061
 P  G  E  I  E  P  I  P  E  D  S  D  S  D  P  G  S  D  S  G  S  D  S  N  S  D  S  G  S  D  S  G  S  D  S  T  S   587
```

FIG. 2A-2

```
AGATAGTGGTTCAGATTCAGCGAGTGATTCAGATTCAGCAAGTGATTCAGACTCAGCGAGTGATTCAGATTCAGCAAGCGATTCCGACTCAGCGAGCGATTCCGACTCAG  2171
 D  S  G  S  D  S  A  S  D  S  D  S  A  S  D  S  D  S  A  S  D  S  D  S  A  S  D  S  D  S  A  S  D  S  D  S  D   624

ACAATGACTCGGATTCAGATAGCGATTCTGACTCAGACAGTGACTCAGATTCCGACAGTGACTCAGATTCAGATAGCGATTCTGACTCAGACAGTGACTCAGATTCAGAT  2281
  N  D  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  D    660

AGCGATTCAGATTCAGATAGCGATTCAGATTCCGACAGTGATTCCGACTCAGACAGCGATTCTGACTCCGACAGTGATTCCGACACAGACAGCGATTCAGATTCCGACAG  2391
 S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S   697

TGATTCCGACTCAGATAGCGATTCCGACTCAGATAGCGACTCAGATTCAGACAGCGATTCAGATTCAGACAGCGATTCAGATTCAGATAGCGATTCAGATTCCGACAGTG  2501
 D  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  D   734

ACTCAGATTCCGACAGTGACTCGGATTCAGATAGCGATTCAGATTCCGACAGTGACTCAGATTCCGACAGTGACTCAGACTCAGACAGTGATTCGGATTCAGCGAGTGAT  2611
  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  A  S  D    770

TCGGATTCAGATAGTGATTCCGACTCCGACAGTGACTCGGATTCAGATAGCGACTCAGACTCGGATAGCGACTCGGATTCAGATAGCGATTCGGACTCAGATAGCGATTC  2721
 S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S   807

AGAATCAGACAGCGATTCAGAATCAGACAGCGATTCAGATTCAGACAGCGACTCAGACAGTGACTCAGATTCAGATAGTGACTCGGATTCAGCGAGTGATTCAGACTCAG  2831
 E  S  D  S  D  S  E  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  D  S  A  S  D  S  D  S  G   844
                                                                                W

GTAGTGACTCCGATTCATCAAGTGATTCCGACTCAGAAAGTGATTCAAATAGCGATTCCGAGTCAGGTTCTAACAATAATGTAGTTCCGCCTAATTCACCTAAAAATGGT  2941
  S  D  S  D  S  S  S  D  S  D  S  E  S  D  S  N  S  D  S  E  S  G  S  N  N  N  V  V  P  P  N  S  P  K  N  G   880
                                                                                M
```

FIG. 2A-3

```
ACTAATGCTTCTAATAAAAATGAGGCTAAAGATAGTAAAGAACCATTACCAGATACAGGTTCTGAAGATGAAGCAAATACGTCACTAATTTGGGGATTATTAGCATCAAT   3051
T  N  A  S  N  K  N  E  A  K  D  S  K  E  P  L  P  D  T  G  S  E  D  E  A  N  T  S  L  I  W  G  L  L  A  S  I    917

AGGTTCATTACTACTTTTCAGAAGAAAAAAAGAAAATAAAGATAAGAAATAAGTAATAATGATATTAAATTAATCATATGATTCATGAAGAAGCCACCTTAAAAAGGTGCT   3161
 G  S  L  L  L  F  R  R  K  K  E  N  K  D  K  K  >                                                                933

TCTTTTACTTGGATTTTCCAAATATATTGTTTGAATATAATTAATAATTAATTCATCAACAGTTAATTATTTTAAAAAGGTAGATGTTATATAATTTGGCTTGGCGAAAA   3271

AATAGGGTGTAAGGTAGGTTGTTAATTAGGGAAAATTAAGGAGAAAATACAGTTGAAAATAAAATTGCTAGTTTTATCATTGGGAGCATTATGTGTATCACAAATTTGGG   3381

AAAGTAATCGTGCGAGTGCAGTGGTTTCTGGGGAGAAGAATCCATATGTATCTGAGTCGTTGAAACTGACTAATAATAAAAATAAATCTAGAACAGTAGAAGAGTATAAG   3491
   HindIII                                                                                                          3499
AAAAGCTT
```

FIG. 2A-4

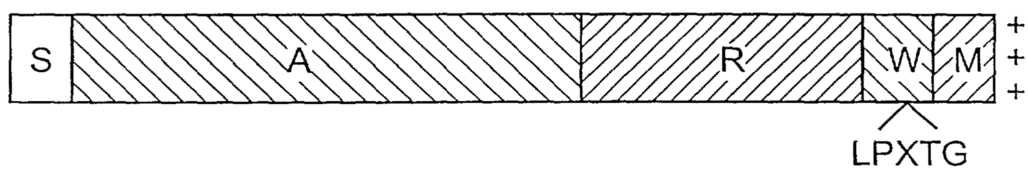
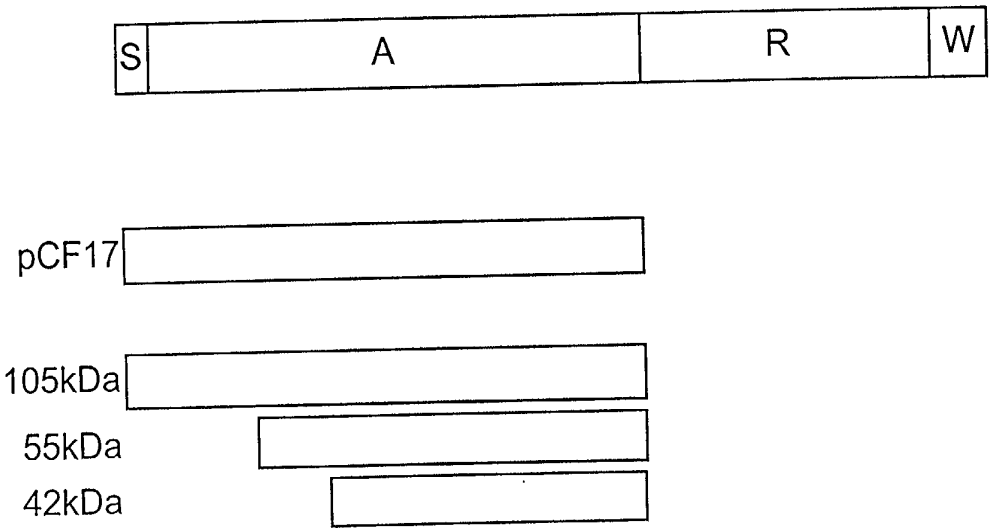
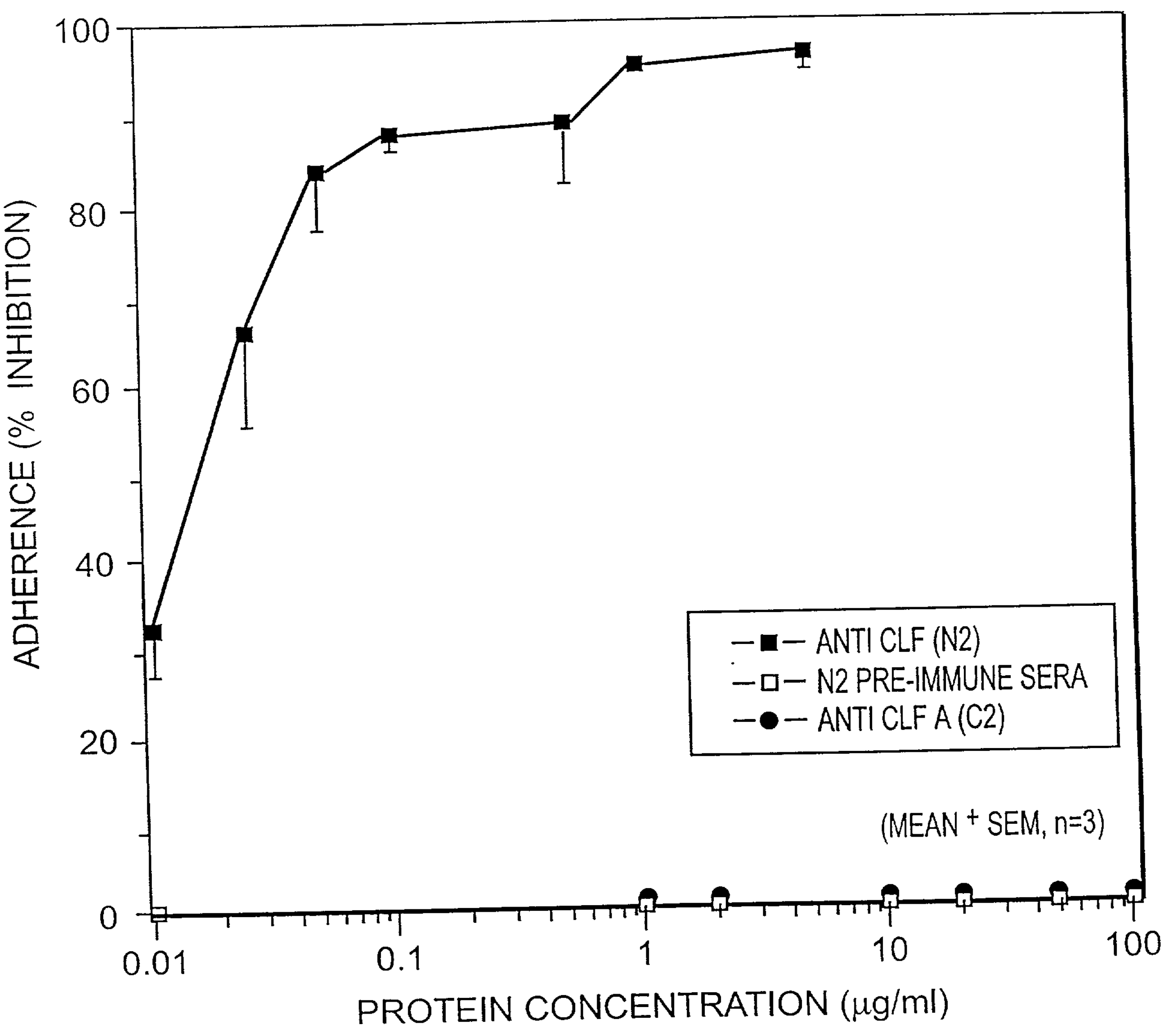


FIG. 2B



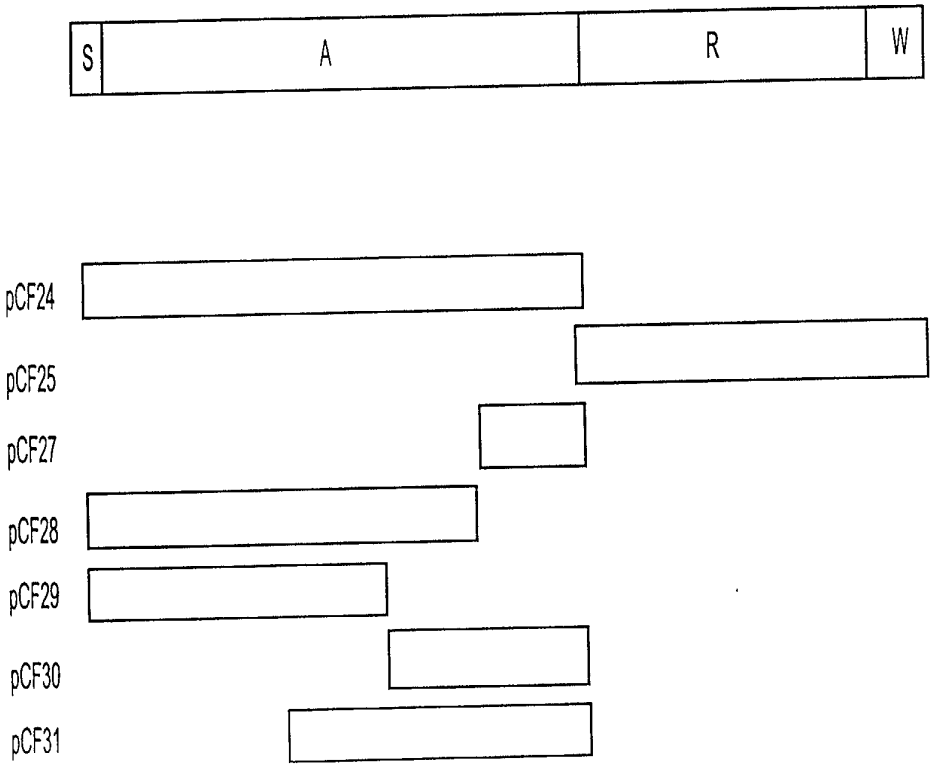
	BINDS Fg	RESIDUES
pCF17	++	23-550
105kDa	++	23-550
55kDa	++	143-550
42kDa	++	219-550

FIG. 3



100
80
60
40
20
0
ADHERENCE (% INHIBITION)
0.01
0.1
1
10
100
PROTEIN CONCENTRATION (μg/ml)
ANTI CLF (N2)
N2 PRE-IMMUNE SERA
ANTI CLF A (C2)
(MEAN + SEM, n=3)

FIG. 4



	BINDS FG	INHIBITS CLUMPING	INHIBIT ADHERENCE	BLOCKS AB	RESIDUES	NUMBER OF RESIDUES
pCF24	++	++	++	++	23-550	527
pCF25	–	–	–	–	546-933	387
pCF27	–	–	–	–	420-550	130
pCF28	–	–	–	+–	23-424	401
pCF29	–	–	–	–	23-308	285
pCF30	–	–	–	++	332-550	218
pCF31	++	++	++	++	221-550	329

FIG. 5

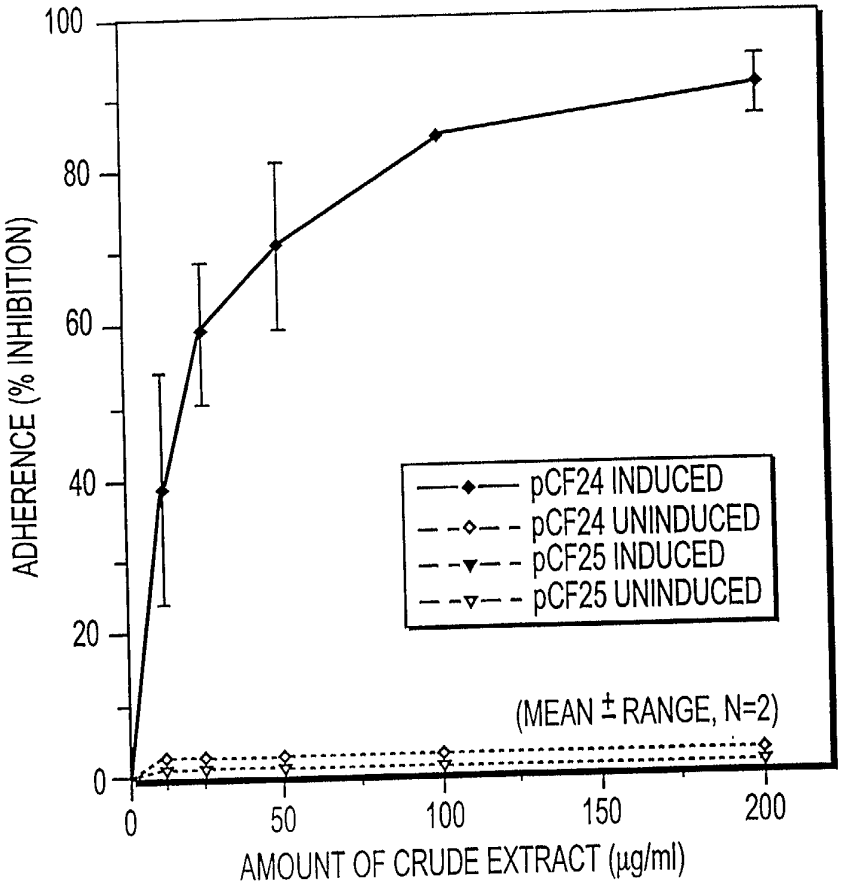

100
80
60
40
20
0
ADHERENCE (% INHIBITION)
pCF24 INDUCED
pCF24 UNINDUCED
pCF25 INDUCED
pCF25 UNINDUCED
(MEAN ± RANGE, N=2)
0
50
100
150
200
AMOUNT OF CRUDE EXTRACT (µg/ml)

FIG. 6A

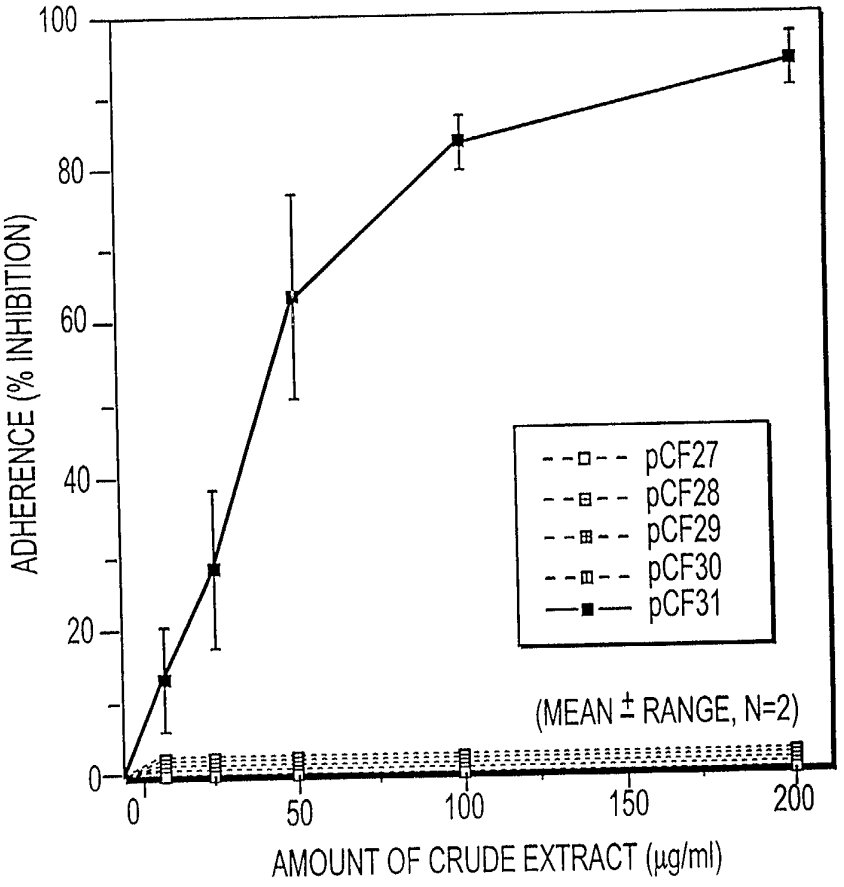

100
80
60
40
20
0
ADHERENCE (% INHIBITION)
pCF27
pCF28
pCF29
pCF30
pCF31
(MEAN ± RANGE, N=2)
0
50
100
150
200
AMOUNT OF CRUDE EXTRACT (µg/ml)

FIG. 6B

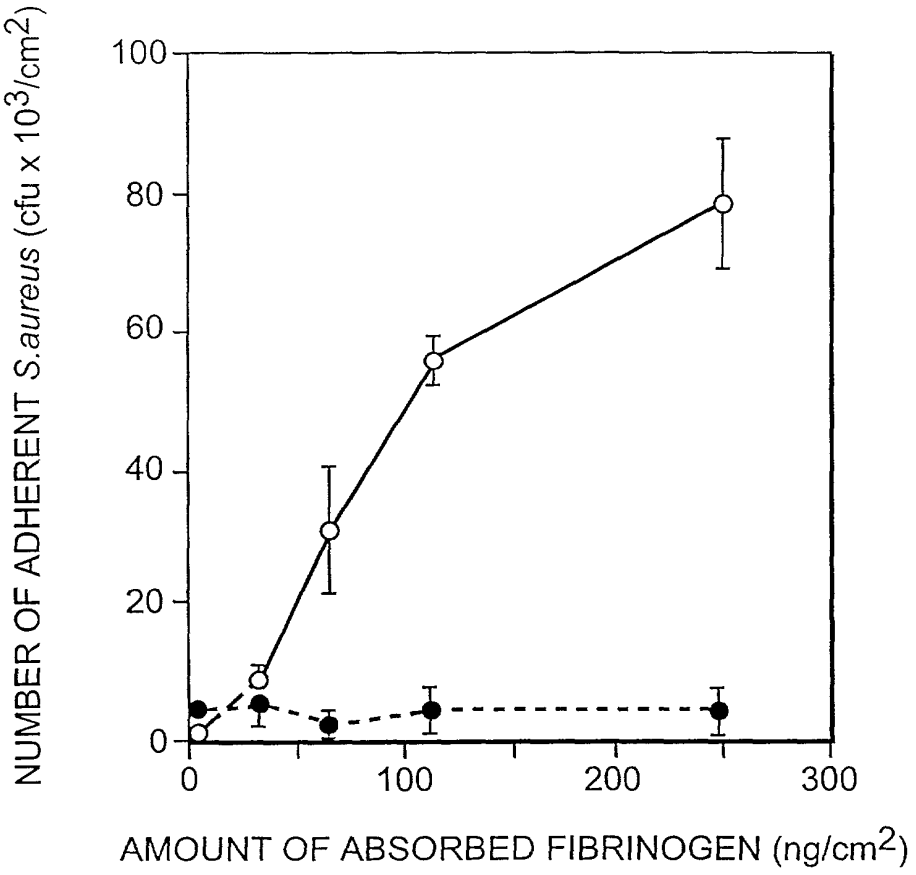

NUMBER OF ADHERENT S.aureus (cfu x 10^3/cm^2)
100
80
60
40
20
0
0
100
200
300
AMOUNT OF ABSORBED FIBRINOGEN (ng/cm^2)

FIG. 7

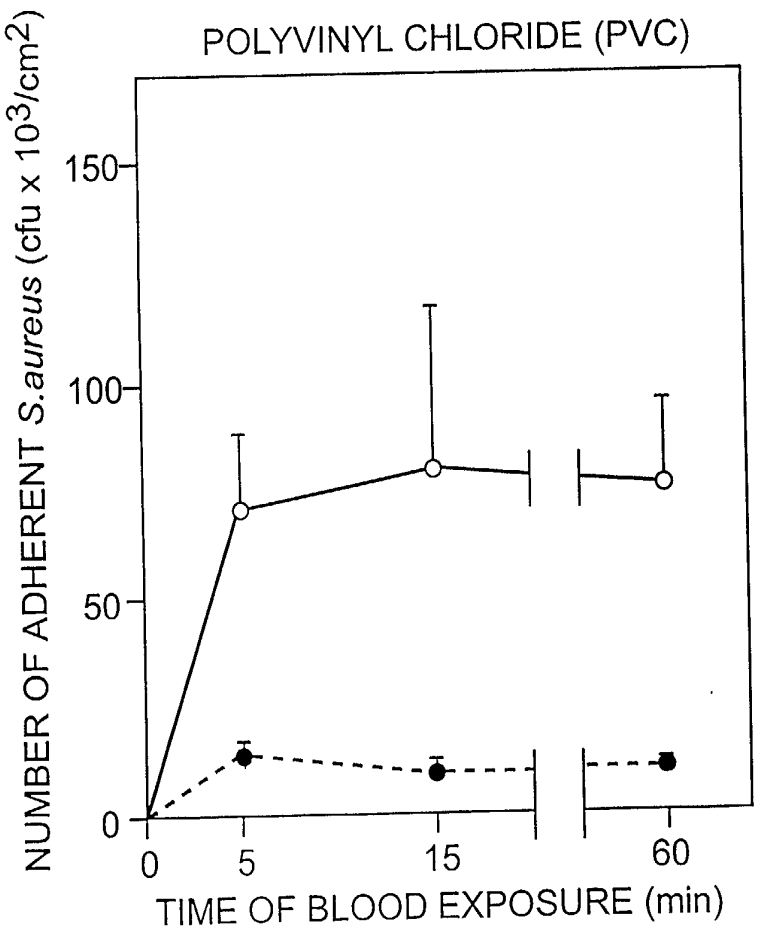

POLYVINYL CHLORIDE (PVC)
NUMBER OF ADHERENT S.aureus (cfu x 10³/cm²)
150
100
50
0
0
5
15
60
TIME OF BLOOD EXPOSURE (min)

FIG. 8A

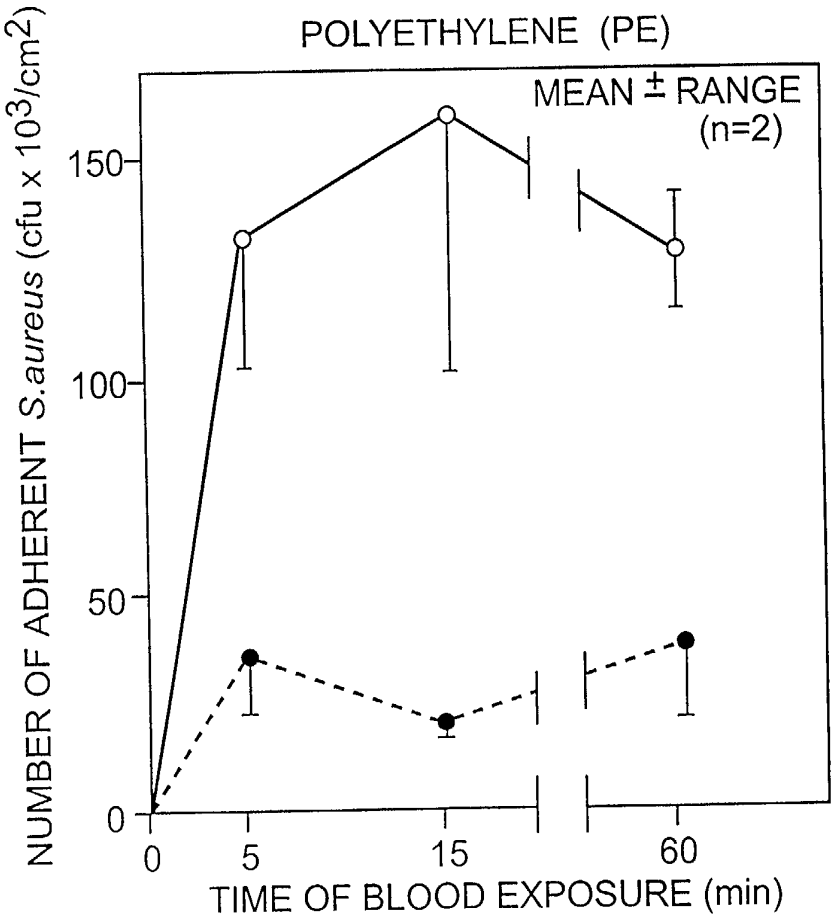

POLYETHYLENE (PE)
MEAN ± RANGE
(n=2)
NUMBER OF ADHERENT S.aureus (cfu x 10³/cm²)
150
100
50
0
0
5
15
60
TIME OF BLOOD EXPOSURE (min)

FIG. 8B

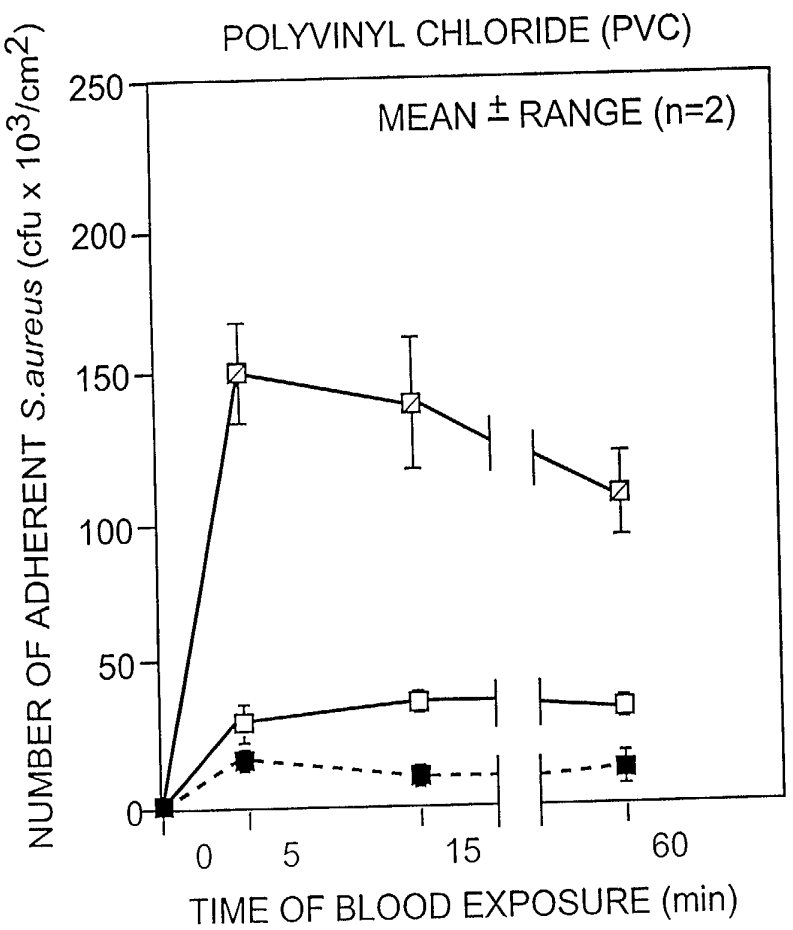

POLYVINYL CHLORIDE (PVC)
MEAN ± RANGE (n=2)
NUMBER OF ADHERENT S.aureus (cfu x 10³/cm²)
250
200
150
100
50
0
0
5
15
60
TIME OF BLOOD EXPOSURE (min)

FIG. 9A

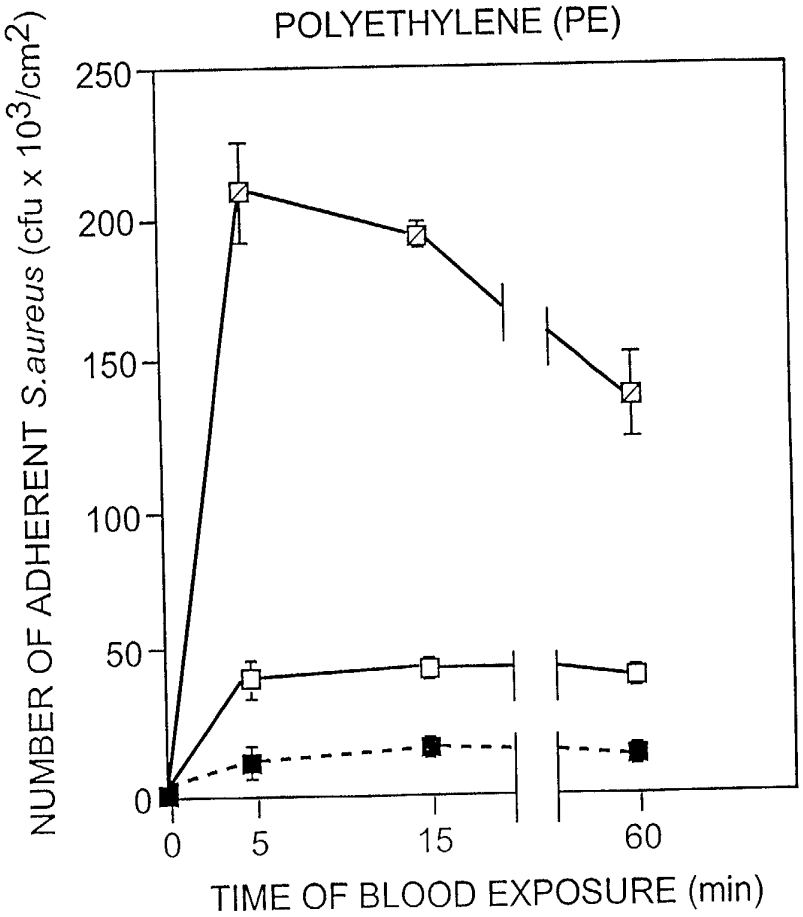

POLYETHYLENE (PE)
NUMBER OF ADHERENT S.aureus (cfu x 10³/cm²)
250
200
150
100
50
0
0
5
15
60
TIME OF BLOOD EXPOSURE (min)

FIG. 9B

UTILITY PATENT
OR DESIGN
SOLE OR JOINT

ATTORNEY'S DOCKET NO

ER, CA[illegible]OR, MUELLER & PLA[illegible]R
UNITED[illegible]TES LETTERS PATENT
DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I declare that I believe I am the original, first and sole inventor if only one name is listed at item 201 below, or a joint inventor if plural names are listed below at items 201 et. seq. of subject matter which is claimed and for which a patent is sought for

the invention entitled THE S.AUREUS FIBRINOGEN BINDING PROTEIN GENE

which is described and claimed in

☑ the attached specification ☐ the specification in application Serial No. . . . filed . . . .
(for declaration not accompanying application papers)

and (if applicable) amended on . . . .

☐ international (PCT) application No. . . filed . . . and as amended on . . . (if any)

I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56

I hereby claim the benefit of priority, under Title 35, United States Code, §119 of any foreign application(s) for patent or inventors certificate having a filing date before that of the application for which priority is claimed.

I hereby claim the benefit, under Title 35, United States Code, §120, of any U.S. application(s) listed in item 105 below. If this application is a continuation-in-part, insofar as the subject matter of any of the claims thereof is not disclosed in the prior U.S. application(s) identified in item 105 below in the manner provided by the first paragraph of Title 35, United States Code, §112. I acknowledge the duty to disclose all information known to me to be material to patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior U.S. application(s) identified in item 105 below and the national or PCT international filing date of this application

FOREIGN APPLICATION(S), IF ANY, FILED WITHIN 12 (6 if a Design) MONTHS PRIOR TO THE FILING DATE OF THIS APPLICATION THE PRIORITY OF WHICH WHERE PERMITTED IS HEREBY CLAIMED UNDER 35 U.S.C. §119

103	COUNTRY	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED YES	NO

104	THIS APPLICATION IS A: ☐ CONTINUATION ☐ DIVISION ☐ CONTINUATION-IN-PART OF PRIOR U.S. APPLICATION	SERIAL NO	FILED

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith

105			
	HAROLD C. WEGNER, Registration No. 25,258	HERBERT I. CANTOR, Registration No. 24,392	HELMUTH A. WEGNER, Registration No. 17,033
	FRANKLIN D. WOLFFE, Registration No. 19,724	DOUGLAS P. MUELLER, Registration No. 30,300	WILLIAM E. PLAYER, Registration No. 31,409

106	SEND CORRESPONDENCE TO	DIRECT TELEPHONE CALLS TO
	WEGNER, CANTOR, MUELLER & PLAYER P.O. Box 18218 Washington, D.C. 20036-8218	202-887-0400

The inventor(s) name must include at least one unabbreviated first or middle name

201				
	FULL NAME OF INVENTOR	LAST NAME: FOSTER	FIRST NAME: TIMOTHY	MIDDLE NAME: JAMES
	RESIDENCE & CITIZENSHIP	CITY OR OTHER LOCATION: DUBLIN	STATE OR COUNTRY: IRELAND	CITIZENSHIP: UNITED KINGDOM
	POST OFFICE ADDRESS	POST OFFICE ADDRESS: 70 COOLAMBER PARK, TEMPLEOGUE, DUBLIN 16, IRELAND		
202	FULL NAME OF INVENTOR	LAST NAME: McDEVITT	FIRST NAME: DAMIEN	MIDDLE NAME: LEO
	RESIDENCE & CITIZENSHIP	CITY OR OTHER LOCATION: DUBLIN	STATE OR COUNTRY: IRELAND	CITIZENSHIP: IRISH
	POST OFFICE ADDRESS	POST OFFICE ADDRESS: 4 DEANS COURT, CHRISTCHURCH SQUARE, DUBLIN 8, IRELAND		
203	FULL NAME OF INVENTOR	LAST NAME	FIRST NAME	MIDDLE NAME
	RESIDENCE & CITIZENSHIP	CITY OR OTHER LOCATION	STATE OR COUNTRY	CITIZENSHIP
	POST OFFICE ADDRESS	POST OFFICE ADDRESS		

Fourth (and more) coinventors on page 2

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine . . . imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon

SIGNATURE OF INVENTOR 201	SIGNATURE OF INVENTOR 202	SIGNATURE OF INVENTOR 203
Timothy James Foster	Damien Leo McDevitt	
DATE: JULY 29, 1994	DATE: JULY 29, 1994	DATE

SEQUENCE LISTING

<110> Foster, Timothy J.
McDevitt, Damien L.

<120> The S. aureus Fibrinogen Binding Protein Gene

<130> 05344.105011

<140> 08/293,728
<141> 1994-08-22

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<170> PatentIn Ver. 2.0

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attcttaaaa tagcaataaa taaaatgttt gttagtaaag tattattgtg gataataaaa 180

tatcgataca aattaattgc tataatgcaa ttttagtgta taattccatt aacagagatt 240

aaatatatct taaagggtat atagttaata taaaatgact ttttaaaaag agggaataaa 300

atg aat atg aag aaa aaa gaa aaa cac gca att cgg aaa aaa tcg att 348
Met Asn Met Lys Lys Lys Glu Lys His Ala Ile Arg Lys Lys Ser Ile
1 5 10 15

ggc gtg gct tca gtg ctt gta ggt acg tta atc ggt ttt gga cta ctc 396
Gly Val Ala Ser Val Leu Val Gly Thr Leu Ile Gly Phe Gly Leu Leu
20 25 30

agc agt aaa gaa gca gat gca agt gaa aat agt gtt acg caa tct gat 444
Ser Ser Lys Glu Ala Asp Ala Ser Glu Asn Ser Val Thr Gln Ser Asp
35 40 45

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agc gca agt aac gaa agc aaa agt aat gat tca agt agc gtt agt gct   492
Ser Ala Ser Asn Glu Ser Lys Ser Asn Asp Ser Ser Ser Val Ser Ala
     50                  55                  60

gca cct aaa aca gac gac aca aac gtg agt gat act aaa aca tcg tca   540
Ala Pro Lys Thr Asp Asp Thr Asn Val Ser Asp Thr Lys Thr Ser Ser
 65                  70                  75                  80

aac act aat aat ggc gaa acg agt gtg gcg caa aat cca gca caa cag   588
Asn Thr Asn Asn Gly Glu Thr Ser Val Ala Gln Asn Pro Ala Gln Gln
                 85                  90                  95

gaa acg aca caa tca tca tca aca aat gca act acg gaa gaa acg ccg   636
Glu Thr Thr Gln Ser Ser Ser Thr Asn Ala Thr Thr Glu Glu Thr Pro
            100                 105                 110

gta act ggt gaa gct act act acg aca acg aat caa gct aat aca ccg   684
Val Thr Gly Glu Ala Thr Thr Thr Thr Thr Asn Gln Ala Asn Thr Pro
        115                 120                 125

gca aca act caa tca agc aat aca aat gcg gag gaa tta gtg aat caa   732
Ala Thr Thr Gln Ser Ser Asn Thr Asn Ala Glu Glu Leu Val Asn Gln
    130                 135                 140

aca agt aat gaa acg act ttt aat gat act aat aca gta tca tct gta   780
Thr Ser Asn Glu Thr Thr Phe Asn Asp Thr Asn Thr Val Ser Ser Val
145                 150                 155                 160

aat tca cct caa aat tct aca aat gcg gaa aat gtt tca aca acg caa   828
Asn Ser Pro Gln Asn Ser Thr Asn Ala Glu Asn Val Ser Thr Thr Gln
                165                 170                 175

gat act tca act gaa gca aca cct tca aac aat gaa tca gct cca cag   876
Asp Thr Ser Thr Glu Ala Thr Pro Ser Asn Asn Glu Ser Ala Pro Gln
            180                 185                 190

agt aca gat gca agt aat aaa gat gta gtt aat caa gcg gtt aat aca   924
Ser Thr Asp Ala Ser Asn Lys Asp Val Val Asn Gln Ala Val Asn Thr
        195                 200                 205

agt gcg cct aga atg aga gca ttt agt tta gcg gca gta gct gca gat   972
Ser Ala Pro Arg Met Arg Ala Phe Ser Leu Ala Ala Val Ala Ala Asp
    210                 215                 220

gca ccg gca gct ggc aca gat att acg aat cag ttg acg aat gtg aca  1020
Ala Pro Ala Ala Gly Thr Asp Ile Thr Asn Gln Leu Thr Asn Val Thr
225                 230                 235                 240
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Val Gly Ile Asp Ser Gly Thr Thr Val Tyr Pro His Gln Ala Gly Tyr
245 250 255

gtc aaa ctg aat tat ggt ttt tca gtg cct aat tct gct gtt aaa ggt 1116
Val Lys Leu Asn Tyr Gly Phe Ser Val Pro Asn Ser Ala Val Lys Gly
260 265 270

gac aca ttc aaa ata act gta cct aaa gaa tta aac tta aat ggt gta 1164
Asp Thr Phe Lys Ile Thr Val Pro Lys Glu Leu Asn Leu Asn Gly Val
275 280 285

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Thr Ser Thr Ala Lys Val Pro Pro Ile Met Ala Gly Asp Gln Val Leu
290 295 300

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Ala Asn Gly Val Ile Asp Ser Asp Gly Asn Val Ile Tyr Thr Phe Thr
305 310 315 320

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Asp Tyr Val Asn Thr Lys Asp Asp Val Lys Ala Thr Leu Thr Met Pro
325 330 335

gct tat att gac cct gaa aat gtt aaa aag aca ggt aat gtg aca ttg 1356
Ala Tyr Ile Asp Pro Glu Asn Val Lys Lys Thr Gly Asn Val Thr Leu
340 345 350

gct act ggc ata ggt agt aca aca gca aac aaa aca gta tta gta gat 1404
Ala Thr Gly Ile Gly Ser Thr Thr Ala Asn Lys Thr Val Leu Val Asp
355 360 365

tat gaa aaa tat ggt aag ttt tat aac tta tct att aaa ggt aca att 1452
Tyr Glu Lys Tyr Gly Lys Phe Tyr Asn Leu Ser Ile Lys Gly Thr Ile
370 375 380

gac caa atc gat aaa aca aat aat acg tat cgt cag aca att tat gtc 1500
Asp Gln Ile Asp Lys Thr Asn Asn Thr Tyr Arg Gln Thr Ile Tyr Val
385 390 395 400

aat cca agt gga gat aac gtt att gcg ccg gtt tta aca ggt aat tta 1548
Asn Pro Ser Gly Asp Asn Val Ile Ala Pro Val Leu Thr Gly Asn Leu
405 410 415

aaa cca aat acg gat agt aat gca tta ata gat cag caa aat aca agt 1596
Lys Pro Asn Thr Asp Ser Asn Ala Leu Ile Asp Gln Gln Asn Thr Ser
420 425 430

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Ile Lys Val Tyr Lys Val Asp Asn Ala Ala Asp Leu Ser Glu Ser Tyr
        435                 440                 445

ttt gtg aat cca gaa aac ttt gag gat gtc act aat agt gtg aat att   1692
Phe Val Asn Pro Glu Asn Phe Glu Asp Val Thr Asn Ser Val Asn Ile
    450                 455                 460

aca ttc cca aat cca aat caa tat aaa gta gag ttt aat acg cct gat   1740
Thr Phe Pro Asn Pro Asn Gln Tyr Lys Val Glu Phe Asn Thr Pro Asp
465                 470                 475                 480

gat caa att aca aca ccg tat ata gta gtt gtt aat ggt cat att gat   1788
Asp Gln Ile Thr Thr Pro Tyr Ile Val Val Val Asn Gly His Ile Asp
                485                 490                 495

ccg aat agc aaa ggt gat tta gct tta cgt tca act tta tat ggg tat   1836
Pro Asn Ser Lys Gly Asp Leu Ala Leu Arg Ser Thr Leu Tyr Gly Tyr
            500                 505                 510

aac tcg aat ata att tgg cgc tct atg tca tgg gac aac gaa gta gca   1884
Asn Ser Asn Ile Ile Trp Arg Ser Met Ser Trp Asp Asn Glu Val Ala
        515                 520                 525

ttt aat aac gga tca ggt tct ggt gac ggt atc gat aaa cca gtt gtt   1932
Phe Asn Asn Gly Ser Gly Ser Gly Asp Gly Ile Asp Lys Pro Val Val
    530                 535                 540

cct gaa caa cct gat gag cct ggt gaa att gaa cca att cca gag gat   1980
Pro Glu Gln Pro Asp Glu Pro Gly Glu Ile Glu Pro Ile Pro Glu Asp
545                 550                 555                 560

tca gat tct gac cca ggt tca gat tct ggc agc gat tct aat tca gat   2028
Ser Asp Ser Asp Pro Gly Ser Asp Ser Gly Ser Asp Ser Asn Ser Asp
                565                 570                 575

agc ggt tca gat tcg ggt agt gat tct aca tca gat agt ggt tca gat   2076
Ser Gly Ser Asp Ser Gly Ser Asp Ser Thr Ser Asp Ser Gly Ser Asp
            580                 585                 590

tca gcg agt gat tca gat tca gca agt gat tca gac tca gcg agt gat   2124
Ser Ala Ser Asp Ser Asp Ser Ala Ser Asp Ser Asp Ser Ala Ser Asp
        595                 600                 605

tca gat tca gca agc gat tcc gac tca gcg agc gat tcc gac tca gac   2172
Ser Asp Ser Ala Ser Asp Ser Asp Ser Ala Ser Asp Ser Asp Ser Asp
    610                 615                 620
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aat gac tcg gat tca gat agc gat tct gac tca gac agt gac tca gat   2220
Asn Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
625                 630                 635                 640

tcc gac agt gac tca gat tca gat agc gat tct gac tca gac agt gac   2268
Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
                645                 650                 655

tca gat tca gat agc gat tca gat tca gat agc gat tca gat tcc gac   2316
Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
            660                 665                 670

agt gat tcc gac tca gac agc gat tct gac tcc gac agt gat tcc gac   2364
Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
        675                 680                 685

tca gac agc gat tca gat tcc gac agt gat tcc gac tca gat agc gat   2412
Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
    690                 695                 700

tcc gac tca gat agc gac tca gat tca gac agc gat tca gat tca gac   2460
Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
705                 710                 715                 720

agc gat tca gat tca gat agc gat tca gat tcc gac agt gac tca gat   2508
Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
                725                 730                 735

tcc gac agt gac tcg gat tca gat agc gat tca gat tcc gac agt gac   2556
Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
            740                 745                 750

tca gat tcc gac agt gac tca gac tca gac agt gat tcg gat tca gcg   2604
Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Ala
        755                 760                 765

agt gat tcg gat tca gat agt gat tcc gac tcc gac agt gac tcg gat   2652
Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
    770                 775                 780

tca gat agc gac tca gac tcg gat agc gac tcg gat tca gat agc gat   2700
Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
785                 790                 795                 800

tcg gac tca gat agc gat tca gaa tca gac agc gat tca gaa tca gac   2748
Ser Asp Ser Asp Ser Asp Ser Glu Ser Asp Ser Asp Ser Glu Ser Asp
                805                 810                 815
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agc gat tca gat tca gac agc gac tca gac agt gac tca gat tca gat   2796
Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
            820                 825                 830

agt gac tcg gat tca gcg agt gat tca gac tca ggt agt gac tcc gat   2844
Ser Asp Ser Asp Ser Ala Ser Asp Ser Asp Ser Gly Ser Asp Ser Asp
        835                 840                 845

tca tca agt gat tcc gac tca gaa agt gat tca aat agc gat tcc gag   2892
Ser Ser Ser Asp Ser Asp Ser Glu Ser Asp Ser Asn Ser Asp Ser Glu
    850                 855                 860

tca ggt tct aac aat aat gta gtt ccg cct aat tca cct aaa aat ggt   2940
Ser Gly Ser Asn Asn Asn Val Val Pro Pro Asn Ser Pro Lys Asn Gly
865                 870                 875                 880

act aat gct tct aat aaa aat gag gct aaa gat agt aaa gaa cca tta   2988
Thr Asn Ala Ser Asn Lys Asn Glu Ala Lys Asp Ser Lys Glu Pro Leu
                885                 890                 895

cca gat aca ggt tct gaa gat gaa gca aat acg tca cta att tgg gga   3036
Pro Asp Thr Gly Ser Glu Asp Glu Ala Asn Thr Ser Leu Ile Trp Gly
            900                 905                 910

tta tta gca tca ata ggt tca tta cta ctt ttc aga aga aaa aaa gaa   3084
Leu Leu Ala Ser Ile Gly Ser Leu Leu Leu Phe Arg Arg Lys Lys Glu
        915                 920                 925

aat aaa gat aag aaa taagtaataa tgatattaaa ttaatcatat gattcatgaa   3139
Asn Lys Asp Lys Lys
    930

gaagccacct taaaaggtgc ttcttttact tggattttcc aaatatattg tttgaatata 3199

attaataatt aattcatcaa cagttaatta ttttaaaaag gtagatgtta tataatttgg 3259

cttggcgaaa aaatagggtg taaggtaggt tgttaattag ggaaaattaa ggagaaaata 3319

cagttgaaaa ataaattgct agttttatca ttgggagcat tatgtgtatc acaaatttgg 3379

gaaagtaatc gtgcgagtgc agtggtttct ggggagaaga atccatatgt atctgagtcg 3439

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Gly Val Ala Ser Val Leu Val Gly Thr Leu Ile Gly Phe Gly Leu Leu
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Ser Ser Lys Glu Ala Asp Ala Ser Glu Asn Ser Val Thr Gln Ser Asp
         35                  40                  45

Ser Ala Ser Asn Glu Ser Lys Ser Asn Asp Ser Ser Ser Val Ser Ala
     50                  55                  60

Ala Pro Lys Thr Asp Asp Thr Asn Val Ser Asp Thr Lys Thr Ser Ser
 65                  70                  75                  80

Asn Thr Asn Asn Gly Glu Thr Ser Val Ala Gln Asn Pro Ala Gln Gln
                 85                  90                  95

Glu Thr Thr Gln Ser Ser Ser Thr Asn Ala Thr Thr Glu Glu Thr Pro
            100                 105                 110

Val Thr Gly Glu Ala Thr Thr Thr Thr Thr Asn Gln Ala Asn Thr Pro
        115                 120                 125

Ala Thr Thr Gln Ser Ser Asn Thr Asn Ala Glu Glu Leu Val Asn Gln
    130                 135                 140

Thr Ser Asn Glu Thr Thr Phe Asn Asp Thr Asn Thr Val Ser Ser Val
145                 150                 155                 160

Asn Ser Pro Gln Asn Ser Thr Asn Ala Glu Asn Val Ser Thr Thr Gln
                165                 170                 175

Asp Thr Ser Thr Glu Ala Thr Pro Ser Asn Asn Glu Ser Ala Pro Gln
            180                 185                 190

Ser Thr Asp Ala Ser Asn Lys Asp Val Val Asn Gln Ala Val Asn Thr
        195                 200                 205

Ser Ala Pro Arg Met Arg Ala Phe Ser Leu Ala Ala Val Ala Ala Asp
    210                 215                 220

Ala Pro Ala Ala Gly Thr Asp Ile Thr Asn Gln Leu Thr Asn Val Thr
225                 230                 235                 240
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Val Gly Ile Asp Ser Gly Thr Thr Val Tyr Pro His Gln Ala Gly Tyr
245 250 255

Val Lys Leu Asn Tyr Gly Phe Ser Val Pro Asn Ser Ala Val Lys Gly
260 265 270

Asp Thr Phe Lys Ile Thr Val Pro Lys Glu Leu Asn Leu Asn Gly Val
275 280 285

Thr Ser Thr Ala Lys Val Pro Pro Ile Met Ala Gly Asp Gln Val Leu
290 295 300

Ala Asn Gly Val Ile Asp Ser Asp Gly Asn Val Ile Tyr Thr Phe Thr
305 310 315 320

Asp Tyr Val Asn Thr Lys Asp Asp Val Lys Ala Thr Leu Thr Met Pro
325 330 335

Ala Tyr Ile Asp Pro Glu Asn Val Lys Lys Thr Gly Asn Val Thr Leu
340 345 350

Ala Thr Gly Ile Gly Ser Thr Thr Ala Asn Lys Thr Val Leu Val Asp
355 360 365

Tyr Glu Lys Tyr Gly Lys Phe Tyr Asn Leu Ser Ile Lys Gly Thr Ile
370 375 380

Asp Gln Ile Asp Lys Thr Asn Asn Thr Tyr Arg Gln Thr Ile Tyr Val
385 390 395 400

Asn Pro Ser Gly Asp Asn Val Ile Ala Pro Val Leu Thr Gly Asn Leu
405 410 415

Lys Pro Asn Thr Asp Ser Asn Ala Leu Ile Asp Gln Gln Asn Thr Ser
420 425 430

Ile Lys Val Tyr Lys Val Asp Asn Ala Ala Asp Leu Ser Glu Ser Tyr
435 440 445

Phe Val Asn Pro Glu Asn Phe Glu Asp Val Thr Asn Ser Val Asn Ile
450 455 460

Thr Phe Pro Asn Pro Asn Gln Tyr Lys Val Glu Phe Asn Thr Pro Asp
465 470 475 480

Asp Gln Ile Thr Thr Pro Tyr Ile Val Val Val Asn Gly His Ile Asp
485 490 495

Pro Asn Ser Lys Gly Asp Leu Ala Leu Arg Ser Thr Leu Tyr Gly Tyr
500 505 510

Asn Ser Asn Ile Ile Trp Arg Ser Met Ser Trp Asp Asn Glu Val Ala
515 520 525

Phe Asn Asn Gly Ser Gly Ser Gly Asp Gly Ile Asp Lys Pro Val Val
530 535 540

Pro Glu Gln Pro Asp Glu Pro Gly Glu Ile Glu Pro Ile Pro Glu Asp
545 550 555 560

Ser Asp Ser Asp Pro Gly Ser Asp Ser Gly Ser Asp Ser Asn Ser Asp
565 570 575

Ser Gly Ser Asp Ser Gly Ser Asp Ser Thr Ser Asp Ser Gly Ser Asp
580 585 590

Ser Ala Ser Asp Ser Asp Ser Ala Ser Asp Ser Asp Ser Ala Ser Asp
595 600 605

Ser Asp Ser Ala Ser Asp Ser Asp Ser Ala Ser Asp Ser Asp Ser Asp
610 615 620

Asn Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
625 630 635 640

Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
645 650 655

Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
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Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
675 680 685

Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
690 695 700

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705 710 715 720

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725 730 735

Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
740 745 750

Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Ala
755 760 765

Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
770 775 780

Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
785 790 795 800

Ser Asp Ser Asp Ser Asp Ser Glu Ser Asp Ser Asp Ser Glu Ser Asp
805 810 815

Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp Ser Asp
820 825 830

Ser Asp Ser Asp Ser Ala Ser Asp Ser Asp Ser Gly Ser Asp Ser Asp
835 840 845

Ser Ser Ser Asp Ser Asp Ser Glu Ser Asp Ser Asn Ser Asp Ser Glu
850 855 860

Ser Gly Ser Asn Asn Asn Val Val Pro Pro Asn Ser Pro Lys Asn Gly
865 870 875 880

Thr Asn Ala Ser Asn Lys Asn Glu Ala Lys Asp Ser Lys Glu Pro Leu
885 890 895

Pro Asp Thr Gly Ser Glu Asp Glu Ala Asn Thr Ser Leu Ile Trp Gly
900 905 910

Leu Leu Ala Ser Ile Gly Ser Leu Leu Leu Phe Arg Arg Lys Lys Glu
915 920 925

Asn Lys Asp Lys Lys
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Ile Asn Lys Met Phe Val Ser Lys Val Leu Leu Trp Ile Ile Lys Tyr
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Arg Tyr Lys Leu Ile Ala Ile Met Gln Phe
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Leu Phe Glu Tyr Asn

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Lys

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